A Longitudinal Study of Closed Head Injury: Neuropsychological Outcome and Structural Analysis using Region of Interest Measurements and Voxel-Based Morphometry

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Abstract

Background: The hippocampus and corpus callosum have been shown to be vulnerable in head injury. Various neuroimaging modalities and quantitative measurement techniques have been employed to investigate pathological changes in these structures. Cognitive and behavioural deficiencies have also been well documented in head injury.

Aims: The aim of this research project was to investigate structural changes in the hippocampus and corpus callosum. Two different quantitative methods were used to measure physical changes and neuropsychological assessment was performed to determine cognitive and behavioural deficit. It was also intended to investigate the relationship between structural change and neuropsychology at 1 and 6 months post injury.

Method: Forty-seven patients with head injury (ranging from mild to severe) had undergone a battery of neuropsychological tests and an MRI scan at 1 and 6 months post injury. T1-weighted MRI scans were obtained and analysis of hippocampus and corpus callosum was performed using region-of-interest techniques and voxel-based morphometry which also included comparison to 18 healthy volunteers. The patients completed neuropsychological assessment at 1 and 6 months post injury and data obtained was analysed with respect to each assessment and with structural data to determine cognitive decline and correlation with neuroanatomy.

Results: Voxel-based morphometry illustrated reduced whole scan signal differences between patients and controls and longitudinal differences between patients which reflected orbitofrontal damage. Using voxel-based morphometry and segmented images, reduced grey matter concentration was found in the patients' hippocampal region when compared to controls. Data from neuropsychological test scores were related to injury severity as measured by the GCS and PTA. Correlations of cognitive test data with the hippocampus, but not the corpus callosum, were present with voxel-based morphometry providing a greater number of associations than region-of-interest analysis. No longitudinal changes were found in the hippocampus or corpus callosum using region-of-interest methodology or voxel-based morphometry.

Conclusions: Decreased grey matter concentration identified with voxel-based morphometry illustrated that structural deficit was present in the head injured patients and does not change between 1 and 6 months. Voxel-based morphometry appears more sensitive for detecting structural changes after head injury than region-of-interest methods. Although the majority of patients had suffered mild head injury, cognitive and neurobehavioural deficits were evident and there were numerous relationships between reduced grey matter concentration and cognitive test scores. The result thus show that subtle effects of diffuse brain damage in the patient group can be detected by neuroimaging and related to cognitive performance.

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Abbreviations

ApoE	Apolipoprotein E
BVRT	Benton Visual Retention Test
COWAT	Controlled Oral Word Association Test
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DAI	Diffuse Axonal Injury
DBM	Deformation Based Morphometry
DNA	Deoxyribo Nucleic Acid
DSM-IV	Diagnostic Statistical Manual for Mental Disorders 4th edition
DTI	Diffusion Tensor Imaging
DWI	Diffusion-Weighted Imaging
EEG	Electro-Encephalogram
fMRI	Functional Magnetic Resonance Imaging
FWHM	Full Width at Half Maximum
GCS	Glasgow Coma Scale
GM	Grey Matter
GOAT	Galveston Orientation and Amnesia Test
GOS	Glasgow Outcome Scale
GOSE	Extended Glasgow Outcome Scale
GP	General Practitioner
HADS	Hospital Anxiety and Depression Scale
ICA	Intracranial Area
LTP	Long Term Potentiation
MCRT	Motor Choice Reaction Time

MEG	Magneto-Encephalogram
MMSE	Mini Mental State Exam
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MSRT	Motor Simple Reaction Time
MTS	Mesial Temporal Sclerosis
NART	National Adult Reading Test
NFI	Neurobehavioural Functioning Inventory
PET	Positron Emission Tomography
PTA	Post Traumatic Amnesia
RAVENS	Regional Analysis Of Volumes Examined In Normalised Space
RTA	Road Traffic Accident
ROI	Region of Interest
SD	Standard Deviation
SDMT	Symbol Digit Modalities Test
SPECT	Single Photon Emission Computed Tomography
SPM	Statistical Parametric Mapping
TBM	Tensor Based Morphometry
TMT	Trail Making Test
VBM	Voxel Based Morphometry
WAIS-R	Weschler Adult Intelligence Scale – Revised
WM	White Matter

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CHAPTER 1: INTRODUCTION

Head Injury

Head injury, defined as brain damage caused by externally inflicted trauma to the head, can result in significant physical, cognitive and behavioural impairment. Improved outcome and survival rates are due in part to quicker and more effective emergency care, specialised treatment facilities and advances in health care provision (Eker et al., 2000). Despite effective preventative measures such as helmet wearing for cyclists and improved vehicle safety, head injury is prevalent throughout different age groups and is a major cause of disability (Thornhill et al., 2000).

As head injury is a major public health problem with recognised problematic cognitive and neurobehavioural impairment, continuing pathological, neuropsychological and pharmacological research regarding the cause and effect of head injury is crucial to develop further understanding and therapeutic intervention. Animal models are important research methods but neuroimaging of the human brain in pathological states is becoming increasingly important allowing in vivo quantification of gross anatomical pathology and exploration at the biochemical and molecular level.

Epidemiology

Head injury is a major public health problem that puts enormous demands on the health care system. In the United Kingdom, an estimated 1 million patients present to hospital each year with head injury representing 10 % of patients attending A&E

departments (Kay & Teasdale, 2001). Of these, 90 % have mild head injury, 5 % have moderate and 5 % have severe head injury. In the region of 20 % require admission for observation and less than 5 % are transferred to neurosurgical care (Kay & Teasdale, 2001). Mortality rates have demonstrated that 9-10 deaths per 100,000 of the UK population were attributed to severe head injury each year in the 1970s (Jennett & MacMillan, 1981).

Although a peak in the age specific incidence of fatal or hospitalised head injury rates is observed in young adulthood (15-30 years), peak A&E presentations due to head injury occur in children under 10 years of age (Brookes et al., 1990). In Scotland, approximately 50 % of head injury presentations to A&E and 20 % of head injury deaths are attributable to children in the 0-14 years age group (Jennet, 1998). There is a higher incidence of head injury in males than in females in most age groups and this is particularly true when assault is the cause of injury (MacCallum et al., 2000; Brookes et al., 2000). This is in part due to a greater number of males participating in high-risk activities.

Causes of Head Injury

The most common causes of head injury normally reported are road traffic accidents (inclusive of motor vehicle occupant, pedestrian and cyclist injuries), falls and assaults. However, the proportion of head injuries as a result of these causes differs between countries (Jennett & MacMillan, 1981) due in part to differences in safety legislation such as road speed limits and cultural norms of behaviour.

Falls

Head injuries as a result of a fall are more common in young children, in adults with alcohol intoxication and the elderly. With older adults, falls are a common cause of head injury, particularly amongst those in institutionalised care, who are more likely to fall and less likely to be able to respond quickly enough to protect the head from injury (Luukinen et al., 1995).

Road Traffic Accidents

Fatality rates due to road traffic accidents in the UK are lower than other developed countries such as Australia, France, Spain and the United States although the overall rate in developed countries has been decreasing since 1968 (Jennett, 1996). The mortality rate in developed countries is decreasing due to measures such as the compulsory wearing of seatbelts and motorcycle helmets, laws on alcohol limits for drivers, reduced speed limits and advanced car safety. Conversely, rates are increasing in developing countries as the rate of traffic increases. In Scotland, RTAs account for almost 50 % of all severe head injuries (Jennett, 1996). Although falls are the most common cause of head injuries in those patients who present to A&E, RTAs are responsible for a disproportionate number of severe head injuries and deaths as illustrated in figure 1.1 (Jennett, 1996).

Assaults

Assaults are more common in younger adults, particularly with males and in economically depressed urban regions. The proportion of injuries due to assaults

varies between countries. For example, from 1 % of males in France to 45 % of males in Johannesburg (Nell & Brown, 1991). Regional variances also exist within countries. In the United States, percentages range from 4 % in Olmsted County, Minnesota to 40 % for inner city black persons in Chicago (Annegers et al., 1980; Whitman, Coonley-Hoganson & Desai, 1984). A study by MacCallum and colleagues of hospitalisation in Scotland between 1990 and 1994 found that 40 % of hospitalisations due to head injury in the age range of 15-34 years was attributed to assault. Higher incidence rates were found for males compared to females for young people of both sexes in deprived areas than their counterparts in more affluent areas (MacCallum et al., 2000).

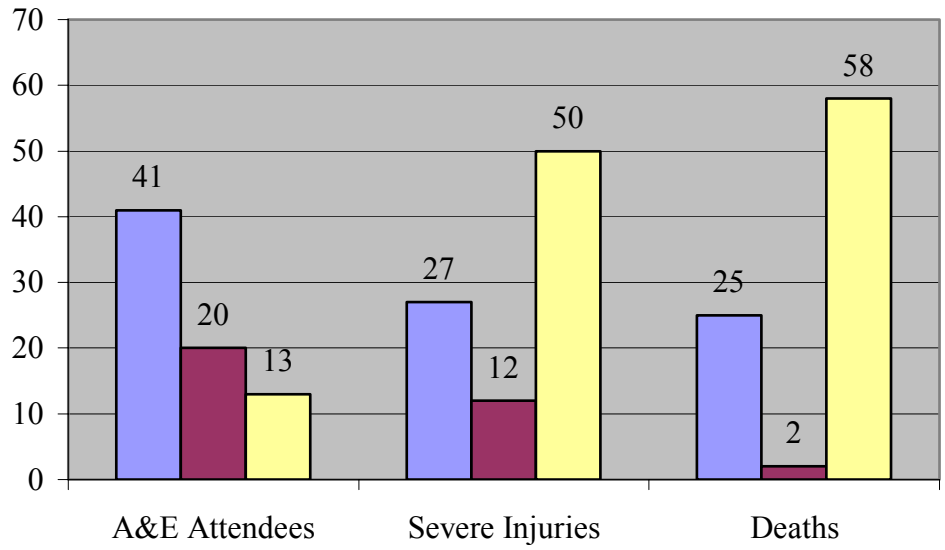


Figure 1.1. Main causes of head injury by severity; severe injuries = coma > 6 hours (adapted from Jennett, 1996)

Sport

Although sport and recreational activities account for a smaller proportion of severe head injuries, a significant number of patients present to A&E as a result of such injuries. Approximately 10 % of admissions in the United States were related to such activities (Whitman, Coonley-Hoganson & Desai, 1984) and in Scotland, 12 % of A&E attendees were attributed to sport (Jennett & MacMillan, 1981).

Alcohol

Falls, assaults and RTAs are often the result of alcohol intoxication. In a large head injured sample (n = 769), 69% of head injuries were involved or thought to have involved alcohol (Thornhill et al., 2000). Alcohol has been demonstrated to have physical and psychological effects. Excessive alcohol consumption at time of injury or a history of moderate to heavy drinking pre-injury revealed increased atrophic changes and cognitive deficits in a head injured group compared to a patient group with head injury but no significant blood alcohol level and zero to mild alcohol use pre-injury (Wilde et al., 2004).

Neuropathology

There are two main categories of brain pathology after trauma, primary and secondary. Primary injury to the brain is a result of mechanical factors that occur at the time of impact and include diffuse axonal injury, diffuse microvascular damage, lacerations of the scalp, skull fractures, haemorrhage and surface contusions and lacerations of the brain. Secondary injury is caused by derangements in

neurophysiology such as raised intracranial pressure, swelling, infection and ischaemia (Graham et al., 2000). Early evidence of the importance of secondary injury came from the 30-40 % of patients with head injury who talk or obey commands before they die suggesting that the degree of primary injury on its own was insufficient to cause death (Reilly et al., 1975). In recent years, there has been an increasing awareness of the pathological cascade of events occurring after head injury and its importance regarding secondary injury (Sahuquillo, Poca & Amorós, 2001). Thus, there is a window of opportunity for possible treatment strategies to be implemented to improve outcome.

Common terminology used to describe pathological insults to the brain includes diffuse and focal injury. Although each can occur separately, in more severe injuries both pathologies coexist and contribute to morbidity (Graham, Gennarelli & McIntosh, 2002). Focal damage includes contusions and haematomas whereas diffuse damage includes diffuse axonal injury (DAI) and diffuse microvascular injury. These changes are often accompanied by generalised abnormalities involving anoxia or hypoxia, widespread neuroexcitation and global and heterogeneous metabolic changes (Povlishock & Katz, 2005). Focal injuries are more likely to be the result of a fall whereas diffuse injuries are more likely to be due to acceleration and deceleration forces commonly associated with RTAs (Adams et al., 1982).

Diffuse Axonal Injury

DAI is caused by rapid rotational acceleration or deceleration forces of adjacent tissues which differ in density, rigidity and cellular architecture. A model proposed

by Ommaya and Gennarelli (1974) suggests that these forces induce mechanical strains that are centripetal in nature initially affecting the surface of the brain and then extending inwards. These shearing forces were thought to result in primary axotomy involving widespread tearing of axons in severe injury. However, in recent times more recognition has been given to the greater importance of secondary processes (Povlishock & Jenkins, 1995). In less severe injury, the mechanical stress impacted on axons ensure that they succumb to a pathological cascade of events which culminate in secondary axotomy over a number of hours and may extend for days or weeks (Blumbergs et al., 1994). This involves structural and functional damage to the axolemma and myelin sheath which leads to the loss of ionic homeostasis and in turn leads to abnormal distribution of ions, swelling of mitochondria, reduction of axonal microtubules and reduced spacing or compaction of axoplasmic neurofilaments. Further disorganisation of the axonal cytoskeleton eventually leads to axonal swellings and finally axonal disconnection (Maxwell, Povlishock & Graham, 1997). In humans, secondary axotomy has been observed 12 hours after initial injury (Christman et al., 1994).

Diffuse Vascular Injury

Diffuse vascular damage results in loss of cerebrovascular autoregulation accompanied by a decreased response to changes in carbon dioxide and perfusion pressure and an initial transient systemic hypertension. Diffuse vascular injury causes death in less than 24 hours and is frequently seen in victims of RTAs where it is associated with severe diffuse axonal injury (Pittella & Gusmão, 2003).

Focal Injury

Focal damage usually involves contusions, haemorrhage, haematomas and/or lesions and the pathophysiology is less complex than that of diffuse injury that extends to axons and dendrites (Gaetz, 2004). Contusions are usually caused by haemorrhagic lesions within the grey matter or at grey-white matter interfaces and neuronal death is usually found at such sites. Contusional injuries typically occur in frontal and temporal regions irrespective of impact site (Gennarelli & Graham, 1998). Subdivisions of contusions include herniation contusions, sliding contusions, coup contusions which occur under the site of injury and contre coup contusions which occur where the brain has made contact with regions distant to, but not always opposite, the site of injury.

Haematoma is a localised collection of blood due to tissue and vascular injury and can be epidural, subdural or intracerebral. Epidural haematomas occur when blood accumulates outwith the dura and are normally caused by the rupture of the middle meningeal artery whereas subdural haematomas are the result of blood accumulation between the dura and the arachnoid due to the rupture of bridging veins. The latter most often occurs after a fall and particularly in the elderly (Howard et al., 1989). Intracerebral haematomas occur within the brain itself and are caused by cerebral contusion. Blood which extends into the arachnoid space between the arachnoid and the brain is known as subarachnoid haemorrhage. Intracerebral haemorrhage occurs within the brain parenchyma secondary to lacerations or contusions and intraventricular haemorrhage tends to occur in very severe head injury. Following contusion or haematoma, blood extends into the adjacent cortex where neurons undergo secondary necrosis due to ischaemia.

Metabolic Changes

Investigation of metabolic changes such as glucose metabolism after brain injury is providing a better understanding of the pathology of head injury. Such changes involve regional, multifocal and/or global irregularities which have been successfully identified due to advances in neuroimaging. Metabolic abnormalities have been found in frontal white matter in head injured patients within the first few weeks of injury (Garnett et al., 2000).

Hypoxia, Ischaemia and Oxygen Free Radicals

Hypoxic brain injury occurs when the brain receives an inadequate supply of oxygen and can result in hypoxic ischaemic brain injury due to a reduced blood flow to the brain caused by a reduction in blood flow or blood pressure. Inflammatory and cytotoxic mechanisms of injury are often the product of ischaemia. Ischaemia may be considered one of the most significant factors related to secondary damage that occurs following brain injury and is caused by hypoxia or impaired cerebral perfusion. The hippocampus is known to be particularly vulnerable to ischaemia (Graham, Gennarelli & McIntosh, 2002) and hypoxia-ischaemia damage to the hippocampus has been associated with memory impairment (Gadian et al., 2000).

Oxygen free radicals are an important factor of cellular pathology after brain injury. These are produced in the early stages of injury both in the central nervous system and elsewhere. Neurons are particularly vulnerable to attack from free radicals and neuronal death occurs due to impaired cellular defences or exposure to excess levels of free radicals (Jesberger & Richardson, 1991).

Brain Swelling

Swelling of the brain can either develop locally in relation to contusions or can be diffuse involving one or more hemispheres. Ischaemia is thought to be the most common underlying pathology in diffuse brain swelling. Lang and associates (1994) found brain swelling after head injury occurred more often in children than in adults and the degree of swelling soon after injury was associated with poor outcome (Lang et al., 1994).

Indices of Injury Severity and Outcome

Predicting long-term recovery after head injury using neurological indices has included measurement of impaired consciousness via scoring on the Glasgow Coma Scale and duration of coma, duration of post traumatic amnesia, papillary reactivity to light and eye movements (Levin et al., 1979). The current study used the Glasgow Coma Scale and duration of post traumatic amnesia as indices of injury severity and the Glasgow Outcome Scale as an assessment of overall outcome.

Post Traumatic Amnesia

Post traumatic amnesia (PTA) refers to the loss of memory for events occurring post injury. When out of coma, length of PTA can be determined by testing the patients' orientation and everyday memory by using a short interview or a standardised questionnaire such as the Galveston Orientation and Amnesia Test (Levin, O'Donnell & Grossman, 1979). Alternatively, PTA can be determined by retrospectively interviewing patients concerning the return of continuous memory

after injury (McMillan, Jongen & Greenwood, 1996). Duration of PTA is considered to be a sensitive predictor of injury severity (Brooks et al., 1986; Bishara et al., 1992) and outcome (Crovitz, Horn & Daniel, 1983; Stuss et al., 1999). PTA has been found to relate to damage in both central and hemispheric structures unlike consciousness related severity measures which have predominantly been found to relate to damage in central structures only (Wilson et al., 1994).

Glasgow Coma Scale

Changes in consciousness such as the level of consciousness and duration of loss of consciousness are the basis of most approaches to classification of severity. The Glasgow Coma Scale (GCS) is the most frequently used measure of consciousness following brain injury and comprises hierarchical levels of response in the areas of eye opening and verbal and motor performance. The summation of the individual categories provides a composite GCS score that has been demonstrated to correlate with outcome following injury (Jennett & Bond, 1975; Levin et al., 1979).

Glasgow Outcome Scale

The Glasgow Outcome Scale (GOS) is an extensively used scale for measuring outcome after traumatic brain injury (Jennett & Bond, 1975) and distinguishes between three classes of conscious survival in terms of handicap; severely disabled, moderately disabled and good recovery although patients assigned to the latter group may not be free of neurological and neuropsychological limitations. The Extended Glasgow Outcome Scale has the addition of upper and lower categories for disability

and recovery (Pettigrew, Wilson & Teasdale, 1998). As the GOS may underestimate the impact of emotional and social factors after head injury, description of outcome is often supplemented with measures such as the Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983) and the Neurobehavioural Functioning Inventory (Kreutzer et al., 1996).

Neuroimaging in Head Injury

The rapid advance of neuroimaging has allowed greater insights into the workings of the normal and abnormal brain and has improved diagnosis, monitoring and treatment in the clinical setting. Until a few decades ago, the standard form of imaging was pneumoencephalography. This technique involved replacing ventricular cerebrospinal fluid with air and provided a two-dimensional view of the brain. Linear measurements of the ventricles were used to calculate a ventricle-to-brain ratio an increase in which signified brain atrophy. However, a single linear measurement in one plane of a two-dimensional image obtained from a three-dimensional brain has limitations of accuracy (Bigler, 2001).

Since the advent of computed tomography (CT) in the 1970s, imaging-based research has advanced in many forms and continues to be employed to investigate the structural, metabolic and functional pathology of head injury and other brain disorders and disease. In the UK, a basic component of risk stratification is radiological assessment of head injured patients. Due to the large number of head injury victims presenting to A&E departments each year, selection of patients for radiological assessment is necessary. CT scans are performed on patients with

impaired conscious levels to identify the presence of mass lesions (Kay & Teasdale, 2001).

It is important to remember that each imaging modality typically addresses one or a few aspects of the neuropathology underlying various diseased brain states. Methods such as electroencephalography relay information about large constellations of neurons whereas other methods such as MRI are specific for brain structure. Techniques that measure haemodynamic responses as a measure of brain function include functional MRI (fMRI), positron emission tomography (PET) and single photon emission computed tomography (SPECT). Structural imaging modalities can be contrasted with functional imaging modalities to provide a more complete assessment of the condition.

Functional Imaging

Greater knowledge of neurobehavioural impairments after head injury may be provided by functional neuroimaging techniques by assessment of regional cerebral blood flow (SPECT) or regional cerebral metabolism whether the patient is inactive (PET) or engaged in various cognitive tasks (PET and fMRI). However, neither PET nor SPECT are used routinely in the acute management of head injury (Azouvi, 2000).

SPECT imaging is performed with a gamma camera to determine regional cerebral blood flow after injection of the tracer 99mTc-HMPAO (hexamethylpropyleneamineoxime). The normal brain demonstrates symmetrical

bilateral tracer distribution with regions of hypoperfusion apparent in head injury. SPECT can detect regions of hypoperfused tissue that appears normal on MRI and has been demonstrated in acute and follow-up imaging after head injury (Mitchener et al., 1997). An association with hypoperfusion and brain atrophy has been demonstrated in mild head injury six months post trauma indicative of secondary ischaemic damage (Hofman et al., 2001). Relationships with cognitive outcome have also been found using SPECT analysis; an association was evident between frontal and medial cortical regions and performance on the Rey Figure test in head injured patients (Stamatakis et al., 1999).

PET has better spatial resolution than SPECT and involves the injection of a radionucleotide to map glucose metabolism, cerebral blood flow and receptor groups. Some studies have suggested that for mild to moderate head injury patients with persistent cognitive or behavioural deficits and normal MRI, PET imaging may be more sensitive for the detection of abnormalities (McAllister et al., 2001b). In a study investigating memory impairment after head injury by Levine and colleagues (2002), the patient group demonstrated higher frontal, occipital and anterior cingulate activity than controls and also showed activation in areas that were not activated in the control group. Thus, this study indicates that after head injury, there are abnormal retrieval responses in memory tasks in moderate to severe head injury as a result of diffuse injury (Levine et al., 2002).

Functional MRI has higher spatial and temporal resolution than other functional modalities and detects alterations in the ratio of cerebral blood oxyhaemoglobin to deoxyhaemoglobin in response to cognitive tasks. McAllister and colleagues

investigated the effects of mild head injury on working memory in patients assessed within one month of injury in comparison to controls. Groups were assessed using tasks with a high working memory load. Both groups displayed fMRI activation in bilateral, dorsolateral, prefrontal and parietal cortices and deterioration of performance in the highest working memory load condition with a greater decline in the patient group. Moreover, the brain activation of patients was smaller than that of controls suggesting that mild head injury results in impairment of working memory or difficulty in the ability to match processing resources to gradations of processing load (McAllister et al., 2001a).

Magnetic Resonance Imaging and its Various Guises

Magnetic resonance imaging (MRI) has advantages over CT such as identifying non-haemorrhagic as well as haemorrhagic lesions and its higher spatial resolution allows detection of smaller white matter lesions. MRI has been shown to detect DAI in mild head injured patients with normal CT scans (Mittl et al., 1994) and acute MRI has been found to detect more abnormalities after head injury than acute CT (Wilson et al., 1988). As MRI allows for quantitative measurement of brain structures, it can also detect DAI indirectly on the basis of tissue loss. However, CT is more widely available, cost effective, has shorter scanning time and is more practical for imaging patients at the acute stage that may be intubated or in traction. For follow-up studies of head injury, MRI is currently the most commonly used structural technique for diagnostic, prognostic and therapeutic objectives.

Improved sensitivity of MRI is the result of advances in MRI and acquisition sequences. Fluid-attenuated inversion-recovery (FLAIR) MRI is a sequence that suppresses the high signal generated from CSF by using a long inversion time. This method has been compared to the T2-weighted spin-echo sequence and has been found to be more sensitive for the detection of lesions and haematomas in head injury (Ashikaga, Araki & Ishida, 1997). Whereas MRI applies radio frequency pulses to protons in water, magnetisation transfer imaging (MTI) applies radio frequency pulses to protons in the macromolecules of tissue allowing for a more sensitive method. This has been demonstrated in mild head injured patients with normal MRI. The magnetisation transfer ratio was lower in the splenium of the corpus callosum in the patient group than in the control group (McGowan et al., 2000).

Magnetic resonance spectroscopy (MRS) detects neurochemical changes that accompany pathological brain states and can detect abnormalities in the brain that may be undetected by conventional MRI. Proton MRS is especially suited to the brain due to the synthesis of N-acetyl aspartate (NAA) in neurons. Using this technique in head injured patients, diffuse pathology in the splenium has been detected (Cecil et al., 1998) and in areas of frontal white matter (Garnett et al., 2000). Diffusion-weighted imaging (DWI) is a rapidly acquired MRI sequence where images are very sensitive to random microscopic motion of water molecules. This is widely used to image cerebral ischaemic stroke where decreased diffusion is associated with cytotoxic oedema and increased diffusion is associated with vasogenic oedema. Abnormalities in DWI occur before abnormalities can be detected using conventional MRI (Garnett, Cadoux-Hudson & Styles, 2001).

Diffusion tensor imaging (DTI) is an extension of DWI where scans comprise a greater number of gradient directions that are applied to estimate the trace of the diffusion tensor. Both DWI and DTI have presented evidence of DAI in head injury in the corpus callosum (Liu et al., 1999; Arfanakis et al., 2002).

MRI is the method of choice for both quantitative and qualitative assessment of the hippocampus and corpus callosum after head injury. Numerous MRI studies of these brain structures have been performed on head injured patients at acute and late stages after injury (Bigler et al., 1997) and in mild, moderate and severe injury in isolation (Tomaiuolo et al., 2004) or in combination (Mathias et al., 2004). Cognitive deficits attributed to these structures have been confirmed using MRI (Bigler et al., 1996) and MRI has been useful in determining recovery of neuropsychological sequelae (Levin et al., 1992) and predicting outcome (Gale et al., 1995).

Outlook for Neuroimaging

Although CT continues to be the primary imaging technique for assessment of acute head injury, specialised MRI structural techniques such as DWI, DTI and MRS and functional modalities such as PET, SPECT and fMRI are greatly increasing the understanding of the neuropathology that underlies head injury and the resulting neuropsychological and neurobehavioural aspects. The advancing science of monitoring gene expression and gene therapy will provide a link between molecular and clinical neuroscience and will allow integration of knowledge among the phenotype, genotype and behaviour in normal and abnormal brain states. These neuroimaging techniques and advances that are on the horizon will continue to prove

important for predicting outcome and channelling the direction of patient treatment and rehabilitation in head injury. Despite such wealth of neuroimaging data, volumetric analysis of MR images is currently still widely used. Specific approaches to analysis of MR data are discussed in detail in chapter four.

The Hippocampus

The hippocampus is one of the oldest phylogenetic structures in the human brain and was named in the 16th century by the anatomist Aranzi, a contemporary of Vesalius, who is thought to have assigned its Greek name due to its resemblance to a seahorse (Scatliff & Clark, 1992). It is a complicated structure that forms part of the limbic system which also includes the subcallosal area, cingulate gyrus and parahippocampal gyrus and exists as a site for integration of a great deal of neural input and output. Detailed descriptions of hippocampal anatomy and complex efferent and afferent hippocampal projections are beyond the scope of this review and the reader is referred to a primary text in neuroanatomy (Kandel, Schwartz & Jessel, 1991).

The Hippocampus and its Role in Memory

The explosion of cognitive interest in the hippocampus began in the 1950s and was due in part to the memory loss of patient H. M. following temporal lobe surgery (Scoville & Milner, 1957). The hippocampus has been investigated in many pathological conditions including epilepsy (Bernasconi et al., 2004), depression (Posener et al., 2003), schizophrenia (Narr et al., 2004), Alzheimer's disease (Jack et

al., 1992), head injury (Tate & Bigler, 2000) and normal aging (Bigler, Anderson & Blatter, 2002).

Before the advent of neuroimaging, theories regarding the location of memory processing were based on neuropathology performed at autopsy on individuals who had memory impairments in life. Disadvantages of such a method include sensitivity of the pathological methods available and reliability of the definition of the amnesic syndrome. Inferences made on the localisation of memory were based on the assumption that the brain had not undergone significant damage between clinical assessment and death, fixation and measurement. Reliable identification of hippocampal pathology has been made possible by modern neuroimaging techniques. MRI is the modality of choice for anatomical assessment of the hippocampus due to the excellent differentiation of grey and white matter that is not surpassed by any other imaging method.

Memory impairment is one of the most common manifestations of brain pathology and is normally one of the earliest symptoms of degenerative brain disease. Memory impairment is one of the deficits that is most likely to persist permanently after brain injury and often has a detrimental effect on social, domestic and work situations (Kapur & Kopelman, 2003). Memory can be broadly classed as implicit or explicit memory. Explicit memory has been generally accepted to involve the hippocampal medial temporal lobe system and implicit memory involves amongst others, the cerebellum and amygdala. In normal conditions, each of these regions are involved in learning to some extent, but each encode a different aspect of the situation (Thompson & Kim, 1996). There are various theories as to the role of the

hippocampus in memory. One popular view states that the hippocampus is where memories are stored before consolidation throughout the cortex (Squire, 1992) whereas another claims that the hippocampus is the site of permanent information storage through multiple memory traces (Nadel & Moscovitch, 1997).

The abundance of evidence that hippocampal integrity is necessary for memory and learning indicates that the hippocampus has plasticity. Synaptic plasticity is a process that involves increasing or decreasing the strength of communication between neurons and is believed to underlie the processing of learning and many forms of long-term memory (Martin, Grimwood & Morris, 2000). Long-term potentiation (LTP) results from rapid excitatory input applied to a depolarised neuron (Giap et al., 2000) and is a popular cellular model for memory. LTP was first demonstrated in the hippocampus by Bliss & Lømo (1973) who discovered that in any of the three major anatomical pathways within the hippocampus, brief trains of high frequency stimulation produced LTP which lasted for days and even weeks in alert animals. Since this initial demonstration of LTP as a memory storage system, LTP has been widely reported to occur in the mammalian hippocampus including humans (Beck et al., 2000). Despite the knowledge that the hippocampus is involved in memory and particularly explicit memory, the exact nature of this involvement is still widely debated. It is outwith the scope of this review to detail the theories regarding the hippocampus and its involvement in learning and memory. Therefore, the reader is referred to Van Petten (2004), Burgess, Maguire & O'Keefe (2002) and Squire (1992).

Hippocampal Vulnerability and Neurogenesis

Experimental models of head injury have provided great insight regarding neuropathology of head injury and the resulting neurobehavioural consequences. They have been instrumental in the development of acute interventions, such as management of intracranial pressure, that have been implemented in clinical practice (Graham et al., 2000). Models of experimental head injury and their attributes that mirror the pathological consequences of human head injury have been comprehensively reviewed (Morales et al., 2005). Each model must fulfill criteria including the ability to produce a quantifiable and reproducible injury and the use of standardised surgical procedures that also include the use of sham animals (Graham et al., 2000).

Hippocampal vulnerability has been demonstrated in experimental studies of head injury and models of cognitive deficit have been proposed. The fluid percussion model is a commonly used model whereby 'concussive' head injury is reproduced and has been used to investigate memory impairment in relation to hippocampal damage in rats and long term outcome (Pierce et al., 1998). Hicks and colleagues used a low level injury designed to replicate mild head injury and was performed in rats after receiving training in the Morris Water Maze. Two days post-injury, the injured rats performed significantly worse on the task than the sham group and hippocampal pathology consisting of neuronal loss and disruption of the blood-brain-barrier was found in the vast majority of the injured rats (Hicks et al., 1993). The same model has been employed in the rat to reproduce severe head injury. Here, injured rats showed spatial and motor deficits when compared to sham controls one year after injury. Neuropathology revealed axonal degeneration of the corpus

callosum and deformation of the hippocampus (Pierce et al., 1998). Hippocampal lesions have also been demonstrated in non-human primates after exposure to mild acceleration-induced injury which resulted in neuronal death possibly due to excitotoxicity (Kotapka et al., 1991).

Experimental head injury models have also been central for the understanding of neurogenesis in the brain. Until recently, it was believed that the adult mammalian central nervous system had very limited regenerative capabilities with no replacement of degenerated neurons. However, that view was challenged four decades ago when experiments revealed neurogenesis in the postnatal rat hippocampus (Altman & Das, 1965). Since then, neurogenesis has been demonstrated in other mammals including tree shrews and marmoset monkeys (Gould et al., 1997; Gould et al., 1998) and it is now generally accepted that neurogenesis in the adult mammalian brain occurs within the sub-ventricular zone and the dentate gyrus of the hippocampus where new neurons, astrocytes and oligodendrocytes are generated (Gage, 2000).

With regards head injury, evidence of hippocampal neurogenesis has come from animal models. Proliferation of neural precursors after injury have been found in proximal and distal areas to the injury site with greater neurogenesis in the dentate gyrus of injured animals compared to control animals (Kernie, Erwin & Parada, 2001). Dash and colleagues (2001) demonstrated that production of precursor cells was optimal at 3 days after injury and that these cells migrated to and implanted in the granule cell layer. Subsequent labelling with a mature neuronal cell marker at 1 month after injury identified that the cells had accumulated over time and had

matured indicating increased neurogenesis after head injury (Dash, Mach & Moore, 2001). Kleindienst and associates (2005) demonstrated that intraventricular infusion of S100B, a neurotrophic/mitogenic produced by astrocytes, enhanced hippocampal neurogenesis in the rat 5 weeks after injury. In addition, improved cognitive performance was found after S100B infusion suggesting a possible important therapeutic role of S100B in treatment of head injury (Kleindienst et al., 2005).

Animal research has provided a great deal of knowledge regarding neurological injury and repair but despite a wide literature of pathological findings in animal models of head injury, the relevance to humans must be addressed as there are caveats of extrapolating results from animal to human. For example, although the gross development of the brain is similar in rats and humans, the development of specific regions can differ such as the lissencephalic brain of the rat compared to the gyrencephalic brain of the human which may be affected differently in head injury (Turkstra, Holland & Bays, 2003). Other common criticisms include the use of genetically well-defined inbred, male animals, varying temporal responses between species and the inability of animal models to accurately reproduce the complexity of clinical head injury (Tolias & Bullock, 2003). However, specific animal models have been developed to represent specific characteristics of human head injury and allow a range of severity (Morales et al., 2005) and parallels between animal and human studies have been found. For example, hippocampal pyramidal cell loss in humans after head injury reflected findings from animal studies providing evidence that hippocampal cell loss in animal models is also found in the head injured patient (Maxwell et al., 2003). Although animal models can neither confirm nor refute hypotheses regarding human pathology after head injury, they can offer insights into

the direction of hypotheses that are relevant to humans and help develop neuroprotective and therapeutic interventions.

Plasticity of the mature central nervous system has been demonstrated in the adult human hippocampus (Eriksson et al., 1998). Postmortem brain tissue was obtained from cancer patients who had been given the drug bromodeoxyuridine between 3 weeks and 2 years before death. This drug is a chemical tracer that is absorbed by dividing cells and is used clinically to monitor proliferation of cancer cells by acting as a marker for mitosis. It was discovered that in all patients, progenitor cells had divided and had continued to divide in the dentate gyrus of the hippocampus until death. Thus, suggesting that the hippocampus retains its ability to generate neurons throughout life (Eriksson et al., 1998). Evidence of neurogenesis in the neocortex of primates (Gould & Reeves, 1999) has implications for the human neocortex – the largest and most complex part of the human brain.

Increased understanding of neurogenesis in the adult brain may lead to cell replacement treatment after injury and in neurodegenerative diseases such as Parkinson's and Alzheimer's. Replacement therapies are based on the introduction of new cells into the damaged area that can differentiate and integrate in the area to replace the function of the damaged cells. Theoretically, this can be achieved by localised anatomical integration whereby new cells receive afferent input, form axonal projections and become involved in neurotransmission. Alternatively, new cells could produce appropriate neurotransmitters and growth factors to aid the survival of existing neurons (Emsley et al., 2004; Björklund & Lindvall, 2000). Transplantation studies have provided evidence that the fate of stem cells from

different brain regions is not restricted by intrinsic programs but are regulated by extrinsic environmental influences such as neurotrophic growth factors (Lie et al., 2004). For a review of environmental and molecular factors and their influence on stem cells the reader is referred to Watts et al. (2005) and Hagg (2005). The potential of neurogenesis in the hippocampus has implications for disorders such as epilepsy that affect this structure and also for treatment after trauma where the hippocampus has been shown to be vulnerable to the effects of head injury.

The Hippocampus and Head Injury in Humans

Disorders of memory are common in head injury (e.g. Bigler et al., 1996) and although many questions remain unanswered regarding the precise nature of the neuroanatomical pathology that underlies memory impairment, implication of the limbic system is accepted. The medial temporal lobe is susceptible to mechanical forces in head injury due to its position in the middle cranial fossa. In addition to deformation caused by mechanical forces, temporal lobe limbic structures such as the hippocampus are exposed to further damage due to exocitotoxic reactions leading to cell death (White & Reynolds, 1996; Gennarelli, Thibault & Graham, 1998). Secondary localised damage such as vascular changes may contribute to further damage and postacutely, hippocampal neurons can succumb to deafferentation and/or de-efferentation causing transneuronal degeneration resulting in cell death (Gennarelli, Thibault & Graham, 1998).

Various studies have investigated the post-traumatic effects of memory in relation to hippocampal atrophy. Bigler and colleagues (1996) have examined hippocampal

memory deficits in patients imaged at early and late intervals post injury. A study to determine the effects of localised structural damage and non-specific neuropathologic effects on memory impairment was performed using patients imaged with MRI within 90 days of injury (mean GCS = 8.1) and after 90 days (mean GCS = 8.6). Hippocampal volume was assessed in relation to memory impairment using savings scores obtained for the Rey-Osterrieth Complex Figure design and the Logical Memory and Visual Reproduction tests of the Wechsler Memory Scale-Revised. Relationships between hippocampal volume and memory were found to differ between early and late groups; no associations were found in the early group but the absolute volume of the left hippocampus was found to relate to each of the three savings indices and the right hippocampus to two indices (Bigler et al., 1996). This demonstrates that pathological changes to brain-behaviour relationships are time dependent. This was also the conclusion of another report investigating neuropsychological effects of head injury and injury chronicity (Wilson et al., 1988).

Temporal horn dilation of the lateral ventricular system is often reported after head injury (Bigler et al., 1997; Blatter et al., 1997). Temporal horn dilation is often regarded as an indirect sign of hippocampal atrophy but the exact mechanisms and relationship between the two remain undetermined. Global functions of memory and intelligence in relation to temporal horn and hippocampal volumes have been investigated in a predominantly severely head injured population divided into two groups, early and late depending on whether they had been imaged up to 100 days before or after head injury. The effect of time dependency after injury was demonstrated as the late group but not the early group, demonstrated a bilateral

reduction in hippocampal volume when compared to controls. Concurrently, temporal horn volume bilaterally increased in both groups when compared to controls and both hippocampal and temporal horn volumes were associated with GCS score.

With regards to indices of memory and intelligence, it was found that temporal horn volume was predictive of intellectual function and hippocampal volume was predictive of verbal memory in a specific interval (71-210 days post trauma) suggesting that these structures may be of clinical use when predicting neurocognitive outcome after head injury (Bigler et al., 1997). Other studies have also examined temporal horn dilation in head injury with regard to neuropsychological deficits and have reported similar conclusions (Gale et al., 1995; Blatter et al., 1997).

Hippocampal output is dependent on the integrity of the fornix which is also vulnerable in head injury and relates to injury severity (Bigler et al., 1997; Gale et al., 1995; Tate and Bigler, 2000). Vulnerability of the fornix is due to its anatomical structure; it comprises long-coursing white matter fibre tracts with half of its length suspended in ventricular space making it susceptible to shearing and rotational effects experienced in head injury. Due to the interconnection of the fornix and hippocampus, it would seem reasonable to assume that there may be an associated interconnection of atrophy following trauma. A study by Tate and Bigler (2000) illustrated that although hippocampal and fornix areas were reduced in comparison to controls, there was only a minimal relationship between the fornix and hippocampus in both groups. Thus, this suggests that hippocampal atrophy does not automatically

predict atrophy of the fornix and vice versa and that volume reduction in each structure is neuropathologically distinct (Tate & Bigler, 2000).

Neuropathological studies add credence to the notion that different pathological mechanisms may be responsible for hippocampal and fornix degeneration. At autopsy, Blumbergs and Scott (1994) found lesions in the fornix but not the hippocampus in fatal mild head injured victims. The patient group had suffered multifocal axonal injury and had later died 2-99 days after injury due to other causes such as pulmonary embolism and pulmonary oedema (Blumbergs & Scott, 1994). Such findings may go some way towards explaining the persistent neuropsychological deficits, particularly memory, often experienced in patients with mild head injury.

Tomaiuolo and colleagues (2004) investigated hippocampal and fornix volume changes in severe head injured patients who were specifically without macroscopically detectable lesions. As mentioned previously, focal lesions may impact on cognitive abilities confounding the analysis of neuropsychological deficit and volume change of a particular structure. In the aforementioned study, patients with a GCS score of <8 were imaged a mean 20 months after injury. Compared to controls, volume reduction in both the hippocampus and fornix were found to have occurred. Volumetric relationships with cognitive measures were also apparent; the right hippocampus was associated with delayed recall of the Rey Figure and the fornix was associated with immediate and delayed recall of word lists and the Rey Figure (Tomaiuolo et al., 2004). Results from studies that have deliberately

eliminated visible lesions avoid possible confounding effects and provide evidence of diffuse damage underlying cognitive deficits.

In addition to the role of the hippocampus in memory, the hippocampus also mediates a preattentive cognitive function known as auditory gating. Auditory gating facilitates selective attention by providing a mechanism for filtering out irrelevant or excessive auditory stimuli. This may be indexed by the P50 evoked waveform to paired auditory stimuli (Adler et al., 1999). Disruption of cholinergic functioning as a result of head injury may lead to diminished control of multimodal gating in the hippocampus leading to abnormal P50 suppression. This has been referred to as the cholinergic hypothesis of attention and memory impairment following head injury (Arciniegas et al., 1999). Further evidence to support this came from a study which investigated impaired auditory gating in conjunction with hippocampal volumetric analysis in head injured, P50-nonsupressing patients. Hippocampal volume loss was greater than that which could be accounted for by total brain volume loss. Thus, providing converging evidence of structural and electrophysiologic abnormalities in the hippocampus of head injured patients (Arciniegas et al., 2001). Voxel-based morphometry has also demonstrated structural abnormalities in head injured patients whereby the pattern of cognitive deficit and structural changes in the basal forebrain, hippocampal formation and neocortex were consistent with a deficit in cholinergic function (Salmond et al., 2005).

Cholinergic systems which project from the basal forebrain to the hippocampus and other areas are an important factor in memory systems and have a role in memory

deterioration in Alzheimer's patients (Francis et al., 1999). Further evidence supporting the cholinergic theory linking acetylcholine to cognitive deficits after head injury comes from pathological studies and findings from pharmacological intervention. Post mortem examination of fatally injured head injured victims has revealed presynaptic cholinergic dysfunction in the neocortex with the presence of haematoma and longer survival rates being associated with lower cholinergic activity (Murdoch et al., 1998). Pike and Hamm (1997) have provided evidence from a fluid percussion model that cognitive deficits after head injury are the result of a decrease in cholinergic neurotransmission and that pharmacological intervention of the muscarinic cholinergic system during the recovery period is seen to improve cognitive outcome. Spatial memory after experimental head injury has shown to be improved by the infusion of nerve growth factor which increased hippocampal cholinergic transmission (Dixon et al., 1997) but as yet, there have only been a few controlled clinical trials to assess pharmacological intervention in human head injury patients. Central acetylcholinesterase inhibitors in the form of drugs such as Donepezil have been effective in improving or stabilising cognitive impairment in Alzheimer's disease (Smith Doody, 2003) and have shown potential for the improvement of memory and attention defecits after injury (Tenovuo, 2005; Khateb et al., 2005). However, there is a great need for large-scale, randomised double blind placebo-controlled trials (Griffin, Van Reekum & Masanic, 2003).

The Corpus Callosum

The corpus callosum has been studied for centuries; it was mentioned by Galen in the second century AD but the first anatomical description was observed by Vesalius in

the 16th century who thought that the primary function of the corpus callosum was one of mechanical support to maintain the integrity of the various cavities. The corpus callosum has been the interest for many pathological and diseased brain states including Alzheimer's disease (Hampel et al., 2002), Schizophrenia (Keshavan et al., 2002), dyslexia (Rumsey et al., 1996), and head injury (Henry-Feugeas et al., 2000).

The corpus callosum is a large commissural bundle of fibres interconnecting the two cerebral hemispheres and is subdivided into the rostrum, genu, body and splenium. The role of the corpus callosum in facilitating hemispheric communication is vital in humans due to the high degree of hemispheric lateralisation in the human brain. A full anatomical description of the corpus callosum and its commissural pathways is outwith the scope of this review. Thus, the reader is referred to a primary text in neuroanatomy (Kandel, Schwartz & Jessel, 1991).

Sub-division of the corpus callosum often involves the distinction of 4 areas, the rostrum, genu, body and splenium. However, it is not unusual to find up to 7 sub-divisions being used or the midsagittal surface area as a single entity. The most widely used system for subdivision was originally proposed by Witelson (1989) and is based on neurohistogical studies. Although the subdivisions use arbitrarily defined boundaries, they provide a good scheme for describing the corpus callosum (Johnson et al., 1996). Inconsistencies in the literature may be the result of using various protocols to divide and measure the corpus callosum sub-divisions as well as methodological discrepancies. The corpus callosum is topographically organised with the anterior sections connecting the frontal regions and the posterior sections

connecting posterior cortical structures. This specialised organisation results in modality-specific areas of the corpus callosum (figure 1.2).

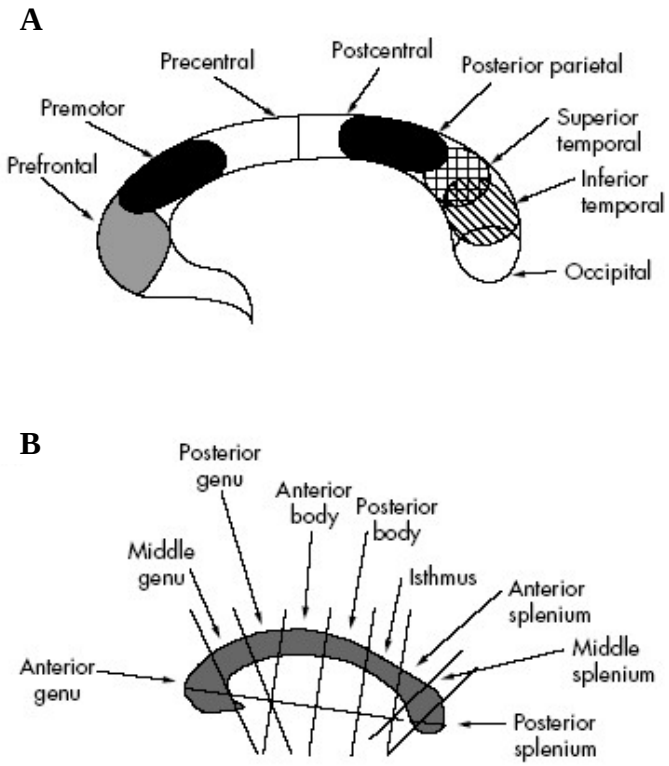


Figure 1.2. Commissural pathways in the corpus callosum based on Witelson's subdivisions (A) and corresponding callosal subdivisions (B). Diagram adapted from Keshavan et al., 2002

The Corpus Callosum and Head Injury

White matter tracts such as the corpus callosum are vulnerable to damage in head injury due to the effects of rotational forces, shearing, tensile effects, axotomy and Wallerian degeneration (Graham, Gennarelli & McIntosh, 2002). This is particularly true for the corpus callosum; its midline location may result in direct contusion due to collision with the falx cerebri and direct trauma due to the fulcrum action which can occur when the cerebral hemispheres rotate in opposite directions to one another.

Furthermore, as frontal and temporal lobe interhemispheric pathways span most of the length of the corpus callosum, damage to these areas in the form of neuronal degeneration is reflected by damage across the length of the corpus callosum (Mathias et al., 2004). Therefore, as the corpus callosum can be directly or indirectly affected by head injury, it can be used as a sensitive indicator of brain atrophy (Johnson et al., 1996).

Due to the superior visualisation and differentiation between grey and white matter, MRI is the preferred imaging modality when examining the corpus callosum. Detailed analysis of location and frequency of lesions have been performed by Kampfl and associates (1998) using MRI in persistent vegetative state patients following head injury. All cases demonstrated DAI and callosal injury while white matter damage in the frontal and temporal lobes was also a common finding. However, the most ubiquitous finding in the persistent vegetative state was a combination of lesions in the corpus callosum and dorsolateral upper brainstem (Kampfl et al., 1998). Although interhemispheric disconnection syndrome after head injury is rare, there are some reports in the literature (e.g. Levin et al., 1989) and disconnection of the corpus callosum along its entire length has been reported (Vuilleumier & Assal, 1995).

It seems that the posterior sections of the corpus callosum are more susceptible to damage after head injury resulting in callosal atrophy as demonstrated with neuroimaging (Benavidez et al., 1999; Cecil et al., 1998; Johnson et al., 1996; Levin et al., 1990a) and also at pathological examination at autopsy (Leclercq et al., 2001). However, anterior sections have also been found to be affected either separately or

accompanied with affected posterior regions (Johnson et al., 1996; Levin et al., 1990a, Takaoka et al., 2002). Furthermore, reports of callosal atrophy practically along the entire callosal length have been documented in 4 out of 16 severe head injured patients with callosal injury. In the remaining patients, lesions were located in isolation or collectively in the rostrum, body, genu and splenium (Takaoka et al., 2002).

Neuropsychological deficit after head injury has been found to relate to atrophy of the corpus callosum (Verger et al., 2001; Benavidez et al., 1999). Mathias and colleagues (2004) used specific visual and tactile reaction time tasks to target posterior sections of the corpus callosum vulnerable to diffuse damage in moderate and severely head injured patients. Areas of damage were found to be located in the regions that serve anterior and posterior areas of the brain and the patient group was impaired on verbal memory and visual and verbal fluency. Slower reaction times on tasks which necessitated the interhemispheric transfer of visual and tactile information were evident but not significant. Furthermore, patients performed disproportionately slower on more demanding tasks suggesting that increased information processing demands had an even greater affect. However, these results also failed to reach significance. It is possible that negative findings for specifically targeted callosal functions are due to reorganisation of interhemispheric pathways after head injury or may be the result of insensitive measurement techniques (Mathias et al., 2004).

These results were mirrored in a similar study which investigated interhemispheric effects on mild head injured patients (Mathias, Beall & Bigler, 2004). Although the

patient group demonstrated deficits in attention, non-verbal fluency and verbal memory and there was some evidence to suggest general slowing, results obtained for visual and tactile reaction time tests did not reach significance suggesting that the damage caused by head injury was not sufficient to cause detrimental effects of information processing. Other explanations include the possibility that transient biochemical disturbances occurred which may resolve over time (Mathias, Beall & Bigler, 2004).

The presence of interhemispheric effects in the early stages after moderate to severe head injury has been demonstrated in a small group of patients (Levander & Sonesson, 1998). Specific language, motor and sensory tests were employed at two different intervals post injury to detect possible disconnections of callosal fibres. The group was found to have a combination of subtle deficits suggestive of mild interhemispheric disconnection although the patients also showed improvement over time (Levander & Sonesson, 1998).

In addition to reduced size of the corpus callosum in head injury, the actual shape has also been reported to differ. A study of severe head injury found that the actual shape of the corpus callosum appeared to be more concave in comparison to a control group. It was also found that length of coma was associated with reduced callosal volume which has been reported previously (Tomaiuolo et al., 2004, Henry-Feugeas et al., 2000) as has other measures of injury severity such as GCS (Gale et al., 1995).

Aims

The aims of the current investigation were:

(1) Longitudinal assessment of structural and neuropsychological changes as a result of head injury. Head injury has been shown to affect the hippocampus and corpus callosum with structural changes being accompanied with a range of cognitive and behavioural deficits such as impaired memory, decreased reaction time and increased depression and anxiety levels. The current study assessed changes between 1 and 5 months post injury, a time span that has previously identified structural changes in the brain and neuropsychological impairment. The rationale behind the selection of the hippocampus and corpus callosum comes from evidence that they are particularly vulnerable to the effects of head injury. Pathological and neuroimaging research has previously shown these structures to be structurally altered following brain trauma.

(2) The implementation of image analysis techniques novel to the study of head injury. In addition to the use of an established method of MR image analysis, namely ROI, more recent techniques using voxel-based morphometry were applied. These techniques have not previously been used to study longitudinal structural changes after head injury and may prove to be more efficient and less biased when determining deficits following brain trauma.

The thesis has three separate methodological chapters containing detailed introductions to the assessments and neuroimaging techniques used. Each of these chapters has a corresponding results chapter encompassing analysed data and a relevant discussion. The final chapter discusses the findings of each methodology in relation to one another and offers conclusions on the investigation as a whole.

CHAPTER 2: NEUROPSYCHOLOGY METHODS

Patient Recruitment

A total of 500 head-injured patients were recruited to the overall project looking at the influence of ApoE genotype on acute damage, recovery rates and neuropsychological outcome after head injury. The 4 cohorts of the study included contusion, neuropsychology, magnetic resonance imaging and monitoring. Of those 500 patients, 117 were used in more than one cohort. However, the actual numbers of patients used in each study were reduced due to various factors such as patients unable and/or unwilling to have a second scan and failing to return for neuropsychological assessment. A buccal swab was taken for DNA analysis using Polymerase Chain Reaction (Markham, 1993) which was performed by the Department of Neuropathology at the Southern General Hospital.

The study was fully approved by the medical ethics committee of the Southern General Hospital in Glasgow.

Figure 2.1 shows the number of patients recruited to each cohort and the number of patients subsequently used. In all but the MRI cohort, the number of patients who actually participated was substantially lower than the number initially recruited. Various reasons accounted for this such as the inability or unwillingness to undergo a second MRI scan and/or failure to complete neuropsychological testing at 1, 3 and 6 months after initial injury.

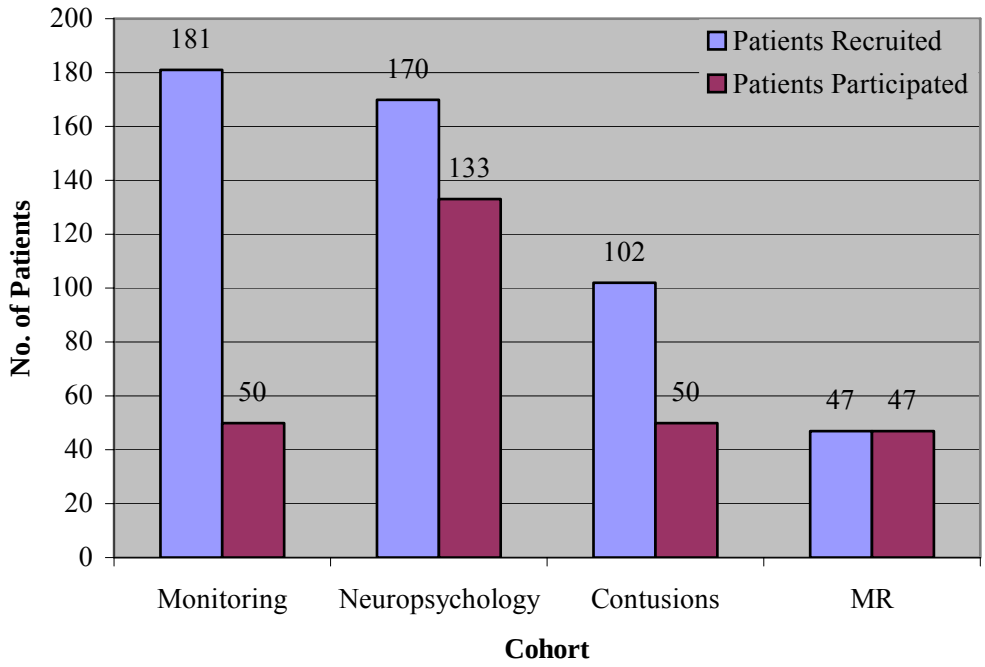


Figure 2.1. Patient recruitment figures

Patient Group Used for Neuropsychology/ROI Measurements

Patients were recruited from admissions to the Neurosurgical Unit at the Southern General Hospital (SGH) in Glasgow 2-4 weeks after injury. Exclusion criteria included age (under 16 or over 75 years), mental handicap, epilepsy, residence distance from SGH (>60 miles), prior intracranial operation, prior head injury requiring neurosurgery, previous hospital treatment for psychiatric illness, prior hospital treatment for alcoholism or drug abuse and dependency due to brain disease pre-injury. Due to the nature of the study and thus the inclusion of an MRI scan, each patient was verified that they did not meet any of the exclusion criteria for MRI i.e. any electronic devices/implants, any metallic fragments in the eyes or any other contra-indications for MRI.

With regard to the present study, only the MR data with corresponding neuropsychology at 1 and 6 months were used. This totalled 47 patients. Patients were invited to attend the Institute of Neurological Sciences or alternatively the research psychologist visited them in their own home to administer the series of neuropsychological tests. The research psychologist was blind to patient genotype and administered neuropsychological testing at 1, 3 and 6 months post injury to investigate aspects of memory, psychomotor ability and outcome 6 months after injury.

The mean age of participants at time of injury was 33.6 years (SD 13.4, range 16-66) and of the 47 patients used in the current study, 4 were female and 43 male. The age distribution and higher number of males in a head-injured population drawn from a city region is not unexpected.

Table 2.1. Age Categories of Patient Group

Age-Group	N	%
16-19	6	12.8
20-29	15	32
30-39	11	23.4
40-49	9	19
50+	6	12.8

Orthopaedic Control Group

An orthopaedic control group was recruited for comparison of neuropsychological test data. This group consisted of 17 patients who had been admitted to hospital following orthopaedic injuries with no injury to the head. Exclusion criteria for admission to the study were the same as for the head-injured patient group. The

mean age of the group at time of injury was 42 years (SD 13.6, range 16-59) and the gender ratio was 12 males: 5 females. Independent t-tests showed that age differences between groups were not significant, $t(61) = 1.8$, $p = 0.09$ and differences on NART error scores were also not significant, $t(61) = 2.1$, $p = 0.06$.

Neuropsychological Testing

A research assistant who was blind to patient genotype performed a battery of neuropsychological tests at 1, 3 and 6 months after injury to investigate aspects of memory, psychomotor ability and outcome at 1 and 6 months after injury. A structured interview at 1-month post injury allowed other information to be collected such as family history and drug/alcohol estimated use and physical limitations both pre and post injury.

The neuropsychological battery of tests consisted of measures designed to investigate aspects of memory, psychomotor ability and outcome 1 and 6 months after injury and are listed in table 2.3. At 1 month, patients may still have been in PTA so it was necessary to include the Galveston Orientation and Amnesia Test. The inclusion of the Mini-Mental State Examination permitted testing of patients who were initially too impaired to complete the full battery of tests. Outcome measures recommended by the National Institute of Neurological Disorders and Stroke Consensus were also undertaken; the National Adult Reading Test, the Extended Glasgow Outcome Scale (GOSE), the Hospital Anxiety and Depression Scale (HADS) and the Neurobehavioural Functioning Inventory (NFI). All subsequent statistical analyses

were carried out using version 11 of SPSS for Windows software (SPSS, Chicago, IL, US).

Table 2.3. Neuropsychological Tests and Assessments Performed

Assessments at 1, 3 and 6 months	Assessments at 6 months only
Galveston Orientation and Amnesia Test	Rey-Osterreith Complex Figure
Rivermead Behavioural Memory Test	Controlled Oral Word Association
Mini-Mental State Examination	Symbol Digit Modalities Test
Digit Span	Trail Making Test A & B
Benton Visual Retention Test	Simple & Choice Reaction Time Tests
Grooved Pegboard using dominant and non-dominant hand	National Adult Reading Test†
Neurobehavioural Inventory†	
Hospital Anxiety and Depression Scale†	
Extended Glasgow Outcome Scale†	

† Outcome measures

Neuropsychological Assessments

National Adult Reading Test (NART)

The National Adult Reading Test (NART) assesses premorbid ability by requiring patients to pronounce 50 words that are phonetically irregular so that only people who have had previous contact with such words can pronounce them correctly. NART scores tend not to decline substantially with age even in dementing individuals (Nelson & McKenna, 1975; Nelson, 1982). It has become the most widely used measure of pre-morbid estimation for various conditions (O'Carroll, 1995) and previous research supports the use of the NART in head injury studies (Watt & O'Carroll, 1999).

Neurobehavioural Functioning Inventory

The Neurobehavioural Functioning Inventory (NFI) is a self-report questionnaire that measures cognitive, emotional, physical, and psychosocial problems both before and after head injury (Kreutzer et al., 1996). The inventory consists of premorbid and postmorbid basic scales which evaluate intrapersonal and interpersonal behaviour. The intrapersonal component evaluates the neurobehavioral functions that rely on internal resources. The interpersonal component reflects the individual's vocational, financial, recreational, and social areas of functioning. Comparison of these responses allows the identification of functional areas that may have been directly affected as a result of injury. As well as a self-report questionnaire, each patient had a nominated relative that was requested to complete the NFI to provide an assessment of the patient from a relative's perspective which may differ from that of the patient.

Glasgow Outcome Scale and Extended Glasgow Outcome Scale

The Glasgow Outcome Scale (GOS) (Jennett & Bond, 1975) is an extensively used scale for measuring outcome after traumatic brain injury and complements the GCS by providing criteria for outcome evaluation. It has 5 categories: 1-death (due to brain damage), 2-persistent vegetative state (absence of cortical function), 3-severe disability (conscious but disabled), 4-moderate disability (disabled but independent) and 5-good recovery (patient appears to revert to pre-injury level of social and career activity). The Extended Glasgow Outcome Scale (GOSE) extends the original 5 GOS categories to 8 namely; 1-dead, 2-vegetative state, 3-lower severe disability, 4-upper severe disability, 5-lower moderate disability, 6-upper moderate disability, 7-

lower good recovery and 8-upper good recovery (Pettigrew, Wilson & Teasdale, 1998). Rating reliability is improved by the provision of such structured interviews. With regards to the current study, the GOSE was collapsed to provide the GOS.

Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983) is a self-rating questionnaire, which measures depression and anxiety in and outside hospital and community settings, avoiding any physical problems, which may be evident. Anxiety and depression are considered as separate parts, each with seven items that are rated from 0 to 3 (most severe) and scores are totalled for each part. A score of <7 is seen as a normal result, a score of 8–10 indicates mild symptoms, 11–14 indicates moderate symptoms and 15+ indicates severe symptoms.

Indices of Injury Severity

Glasgow Coma Scale

The Glasgow Coma Scale (GCS) (Teasdale & Jennett, 1974) is the most widely used method to quantify levels of consciousness following traumatic brain injury. It is extensively used due to its simplicity, its relatively high degree of interobserver reliability and because, to a degree, it correlates with outcome following brain injury (Levin et al., 1979). It is divided into 3 categories; eye opening, verbal response and motor response and a composite coma score results from the addition of the 3 individual scores.

Table 2.4. Categories of the Glasgow Coma Scale

Glasgow Coma Score		
Eye Opening (E)	Verbal Response (V)	Motor Response (M)
4=Spontaneous	5=Normal conversation	6=Normal
3=To voice	4=Disoriented conversation	5=Localises to pain
2=To pain	3=Words, but not coherent	4=Withdraws to pain
1=None	2=No words...only sounds	3=Decorticate posture
	1=None	2=Decerebrate
		1=None
		Total = E+V+M

Table 2.4 illustrates the 3 categories of the GCS and their measured responses with associated scores. With regards to injury severity, a score of 3-8 is deemed severe, 9-12 is moderate and 13-15 is mild.

Although relatively easy to administer, there are limitations. The patient may be intubated and unable to talk or factors which alter a patient's level of consciousness may be problematic in discerning the extent of severity of head injury. Thus, shock, hypoxemia, drug use, alcohol intoxication and metabolic disturbances may in themselves alter the GCS regardless of the head injury.

Post-Traumatic Amnesia

Post-traumatic amnesia (PTA) is the period following head injury when patients are disorientated and acquisition of new information is impaired. The duration of PTA, the interval from the time of injury to the recovery of sequential memory, is considered the most sensitive predictor of injury severity after traumatic brain injury (Brooks et al., 1986; Bishara et al., 1992) as well as the most dependable indication of predicted outcome, even in mild cases (Crovitz, Horn & Daniel, 1983; Stuss et al.,

1999). Estimates of severity based on the duration of PTA generally correspond with the GCS, but it can provide a finer scaling of severity (table 2.5) and has been found to better predict outcome (Lezak, Howieson & Loring, 2004).

Table 2.5. Severity of Injury Based on Duration of Post-Traumatic Amnesia

PTA Duration	Injury Severity
< 5 minutes	Very mild
5 – 60 minutes	Mild
1 – 24 hours	Moderate
1 – 7 days	Severe
1 – 4 weeks	Very severe
> 4 weeks	Extremely severe

Patients participating in the current study were interviewed at 6 months and scored for PTA on a 4-point scale: 1 = No PTA, 2 = 0-24 hours, 3 = >24 hours to 7 days and 4 = >7 days.

Cognitive Tests

Galveston Orientation and Amnesia Test

The Galveston Orientation and Amnesia Test (GOAT) like the Glasgow Coma Scale, was designed for repeated measurements and can be employed as many times as is required. In brain-injured patients, the GOAT is a sensitive indicator of responsivity and also serves as an outcome predictor. It is a short mental status examination designed to evaluate the extent and duration of confusion and amnesia following traumatic brain injury (Levin, O'Donnell & Grossman, 1979). There are a total of 10 questions with 2 specifically relating to amnesia i.e. asking the patient the first and last recollection of events pre- and post injury respectively. The remaining 8

questions are concerned with orientation of time, place and person. A score of 78 or more on three consecutive occasions is considered to indicate that a patient is out of post-traumatic amnesia and in the 'normal range' although amnesic problems can still persevere despite normalisation of orientation.

Rivermead Behavioural Memory Test (Immediate and Delayed Story Recall)

This involves the examiner reading a short story that the patient must recall immediately after presentation and again after a 15-minute interval (Wilson, Cockburn & Baddeley, 1991). The number of correctly remembered details relayed back to the examiner provides the test score. Stories were selected for presentation at at each assessment and the same story was used for all patients at each assessment to avoid introducing a confounding covariate in subsequent correlational analysis.

Mini-Mental State Examination

This is a widely used standardised cognitive screening test first used as a method of ranking cognition (Folstein, Folstein & McHugh, 1975) and now extensively used in the field of dementia. It is a global assessment of orientation to time and place (10 points), registration of 3 words (3 points), attention and calculation (5 points), recall of 3 words (3 points), language (8 points) and visual construction (1 point). Scores range from 25-30 for normals, 21-24 for mild Alzheimer's disease (AD), 14-20 for moderate AD, and <13 in severe AD. The Mini-Mental State Exam (MMSE) has been used to look at disease progression as well as rating areas of functional impairment.

Digit Span

The Digit Span is part of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) and measures attention and short-term working memory (Wechsler, 1981). Orally presented, it is a measure of how many sequential digits can be stored and verbally recalled in the correct order.

The task has 2 components involving repetition of up to 8-digit sequences. With Digits Forward, patients are required to repeat the digit sequences back to the examiner in the order they were presented; the Digits Backward task requires the patients to repeat the digit sequences in the reverse order to which they were presented. Digits Forward and Backward tap distinct but highly interdependent cognitive functions. Digits Forward primarily taps short-term auditory memory while Digits Backward measures the ability to manipulate verbal information while in temporary storage mediated by attention and executive processes (Hale, Hoeppner & Fiorello, 2002). The separate scores in the digit span task allow evaluation of possible dissociation of these functions in head-injured patients.

Benton Visual Retention Test

The Benton Visual Retention Test (BVRT) is designed to assess visual perception, visual memory, and visuoconstructive abilities. It involves the presentation of 10 cards, each consisting of one or more simple geometric designs, for 10 seconds after which, the patient must immediately reproduce the design. The test requires the use of visuospatial conception, verbal conceptualisation immediate recall and visuomotor reproduction and because it involves a number of capacities, it is sensitive to the

presence of brain damage with head injured patients more prone to making errors than matched control subjects (Levin et al., 1990b).

Grooved Pegboard Test

The Grooved Pegboard is a manipulative dexterity test and is part of the Wisconsin Neuropsychological Test Battery (Matthews & Kløve, 1964). The apparatus consists of 25 holes with randomly positioned slots. Pegs with a ridge along one side must be rotated to match the hole for correct insertion. This test requires more complex visual-motor coordination than most pegboard tests. As well as measuring slowing due to medication or disease progression for example, Parkinsonism, it can also aid the evaluation of lateralised brain damage. The patient uses the dominant and non-dominant hand in separate trials and the score reflects the completion time.

Rey-Osterrieth Complex Figure Test

This task is designed to assess perceptual organisation and visual memory by examining the ability to construct a freehand copy of a complex non-verbalisable figure by directly copying it and reproducing it from memory after a period of 3 minutes and without prior notice (Rey, 1941; Osterrieth, 1944). Scoring was based on the widely used 36-point scoring system with scores based on accuracy and placement criteria.

Controlled Oral Word Association Test

The Controlled Oral Word Association Test (COWAT) assesses executive functioning and in particular, word fluency. In this test participants have 60 seconds to state as many words as possible beginning with letters of the alphabet specified by the tester; in this case, the letters F, A and S and the names of animals were used. Word fluency tests which call upon the subject to generate word lists can be indicators of brain dysfunction. Frontal lesions have found to negatively affect fluency scores with lesions located on the left resulting in a more marked decrease in word production than lesions located on the right (Miceli et al., 1981; Perret, 1974).

Symbol Digit Modalities Test

The Symbol Digits Modalities Test (SDMT) was designed for early screening of cerebral dysfunction (Smith, 1982) and is similar to the WAIS Digit Symbol Test. The test measures concentration, rapid decision-making and visual-motor speed and requires patients to match numbers to a series of different symbols presented randomly on a single test page, using a key found at the top. There are 120 matches to be made in total and each subject has to make as many as possible, working in a consistent order, within a 90 second period. Both versions of the test, written and verbal, were performed.

Trail Making Test A & B

These tests measure attention, visual searching, mental processing speed, and the ability to mentally control simultaneous stimulus patterns. These tests are sensitive

to global brain status but are not overly sensitive to minor brain injuries (Reitan, 1994). In part A, the patient has to quickly draw lines on a page to connect 25 consecutive numbers; this requires attention, visual searching, motor coordination and speed. In part B, the patient must draw the lines alternating between numbers and letters. Part B is 56 cm longer and has more visually interfering stimuli than part A. Thus, it is more difficult not only because it is a more difficult cognitive task which also requires mental flexibility and concentration as well as motivation, problem solving, and impulse control, but also because of its increased demands in motor speed and visual search. It is part B which is sensitive to frontal lobe damage.

Motor Reaction Time Tests (Simple and Choice)

Motor Reaction Time tests are designed to assess speed of information processing and fall into two categories, Simple and Choice Reaction Time. In the former, patients were asked to make a single response to a single stimulus presented on a computer screen whereas in the latter, patients had to choose 1 of 2 responses determined by the presented stimulus. Both the speed and accuracy with which responses are made allow inferences about the underlying cognitive processes. For example, reaction time is frequently slowed with brain injury and often underlies attentional deficits (Kløve, 1987; Van Zomeren, Brouwer & Deelman, 1984).

CHAPTER 3: NEUROPSYCHOLOGICAL ASSESSMENT

The patient group was assessed at 1, 3 and 6 months after injury. Epidemiological data and clinical variables are described and clinical indices of severity and outcome measures were evaluated and, where appropriate, were investigated in relation to cognitive measures. Finally, cognitive test scores at each of the three assessment intervals were examined to determine temporal changes in cognitive ability.

Patient Group Used for Neuropsychology/Neuroimaging

Patients were recruited from admissions to the Neurosurgical Unit at the Southern General Hospital in Glasgow. These patients participated in neuroimaging and were drawn from a larger cohort. Assessment was conducted blind to patient genotype using selected neuropsychological tests. These tests were carried out at 1, 3 and 6 months after injury and investigated aspects of memory, psychomotor ability and outcome. The causes of injury are illustrated in figure 3.1. Falls (36 %) and assaults (34 %) were the most common causes of injury with 19 % caused by road traffic accidents, 9 % caused by work-related accidents and 2 % being sports related.

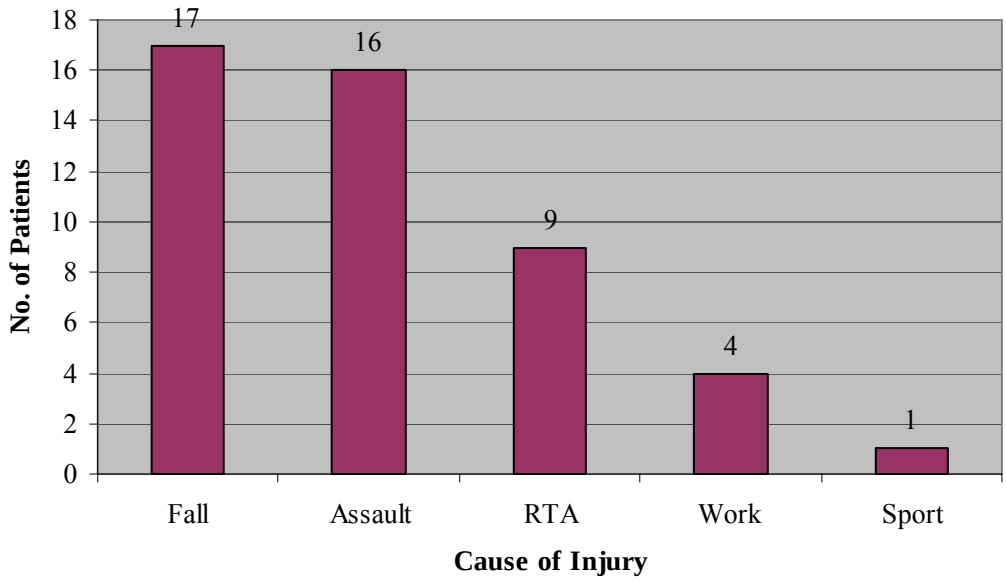


Figure 3.1. Cause of head injury in patient group

Patients were categorised in age bands (table 3.1); the mean age of participants at time of injury was 33.6 years (SD 13.4, range 16-66) and of the 47 patients used in the current study, 4 were female and 43 male.

Table 3.1. Age Categories of Patient Group

Age-Group	N	%
16-19	6	12.8
20-29	15	32
30-39	11	23.4
40-49	9	19
50+	6	12.8

Table 3.2. Mean Age for Cause of Injury

	Fall	Assault	RTA	Work	Sport
Mean Age	37	31	29	39	20

A one-way ANOVA showed no relationship with age and type of injury, $F(46) = 1.3$, $p = 0.28$.

Mean years of education were 11.4 (SD 1.8) ranging from 9 to 17 years for the 45 patients with educational data available. Of the 46 patients with completed marital status, 35 % were married or cohabiting; 6 % were separated, divorced or widowed while the majority, 59 %, were currently not married/cohabiting.

Glasgow Coma Scale

Injury severity was assessed using the Glasgow Coma Scale (GCS) at 2 different stages: on admission to accident and emergency and the worst score during the first 24-hour interval. A third severity classification (GCS & CT) was compiled which followed the conventional GCS at A&E banding except that GCS 13-15 was classed as moderate if there was an abnormal CT scan. This was done on the basis that patients with mild head injury accompanied with acute radiological abnormalities have a less favourable outcome than mild head injury alone (Williams, Levin & Eisenberg, 1990).

Table 3.3. Injury Severity as Measured by the Glasgow Coma Scale

	Glasgow Coma Scale Severity			
	3-8 (Severe)	9-12 (Moderate)	13-15 (Mild)	Missing
A&E	7	4	36	0
24-Hour	10	5	25	7
GCS & CT	7	32	8	0

As shown in table 3.3, for 7 patients the worst 24-hour GCS score could not be ascertained. As stated earlier, patients could not be assessed if they were intubated and/or sedated or consciousness was impaired when the GCS was to be performed. As a result of combining the presence of an abnormal CT scan with the patient's

A&E GCS score to give a GCS & CT score, 28 patients moved from being classed as mild to moderate. Using this categorisation, the majority of patients were classed as moderate (68%), with mild cases providing 17 % of the group and severe cases providing 15 % (percentages given as a proportion of the entire patient group, n = 47). However, as this classification is not widely used, it was not implemented in analysis in the current study to allow for comparison with other research.

Clinical Variables

Table 3.4. Selected Clinical Variables of Patient Group

Variable	N	%	Variable	N	%
Duration of PTA			*Haematoma*		
No PTA	4	8.5	No Haematoma	21	44.7
0 - 24 hours	21	44.7	Subdural	10	21.3
24 hours – 7 days	14	29.8	Extradural	9	19.1
> 7 days	8	17	Intracerebral	1	2.1
			Any region	5	10.6
			Subarachnoid	1	2.1
Skull Fracture			Haemorrhage		
No Fracture	18	38.3	*Surgery*		
Skull Fracture	29	61.7			
			Yes	26	55.3
Contusion			No	21	44.7
No contusions	28	59.6			
Frontal	12	25.5			
Parietal	2	4.2			
Temporal	9	19.1			
Occipital	2	4.2			
>1 Region	5	10.6			

Percentages are given as a proportion of the patient group (n = 47)
PTA = Post Traumatic Amnesia

The duration of PTA was consistent with mild closed-head injury with a median duration of 1 day and a range from 0 to 22 days. Surgery was required in the majority of cases (55 %) with most surgical procedures involving craniotomy and evacuation of haematoma. One patient required repair of the anterior fossa and another required facial reconstruction. Of the patient group, 40 % had 1 or more contusions, 55 % had 1 or more haematomas while 23 % had suffered both.

Clinical Indices of Severity at 1 and 6 Months

Glasgow Coma Scale

Spearman ranked correlations were performed between neuropsychological test scores and GCS scores at 1 and 6-months post injury. Table 3.5 illustrates that severity based on the 24 hour worst GCS significantly correlated with neuropsychological performance more frequently than assessment at initial admission to hospital. For both GCS at A&E and 24 hour worst GCS, all correlations were in the expected direction with regard to neuropsychological performance.

Post Traumatic Amnesia

Associations with test scores were generally consistent with estimated duration of PTA and PTA grouping. PTA associations showed a trend whereby correlational relationships with test scores mirrored the direction of association of GCS with test scores. This was true in all but two associations, delayed recall at 1 month and

BVRT at 6 months. Of the tests that were performed at both 1 and 6 months, the Grooved Pegboard test correlated positively with PTA regardless of handedness (table 3.5) with the association being stronger at 1 month. A positive association with BVRT error scores was found to be significant at 1 month but nonsignificant at 6 months post injury.

Table 3.5. Spearman Correlations Between GCS and Test Scores at 1 and 6 Months

	GCS at A&E	GCS 24 Hr Worst	PTA Grouping
1 Month:			
Immediate Story Recall	.08 (46)	.08 (39)	.4 (47)
Delayed Story Recall	.06 (46)	.08 (39)	-.07 (47)
Digit Span	.1 (46)	.08 (39)	-.21 (47)
BVRT	-.12 (46)	-.16 (39)	.43** (47)
Pegboard: D	-.45** (46)	-.47** (39)	.35** (46)
ND	-.38* (45)	-.51** (39)	.56** (45)
6 Months:			
Immediate Story Recall	.24 (46)	.17 (39)	-.22 (46)
Delayed Story Recall	.15 (46)	.16 (39)	-.22 (46)
Digit Span	.01 (46)	.01 (39)	-.22 (46)
BVRT	-.15 (46)	-.01 (39)	.08 (46)
Pegboard: D	-.26 (46)	-.28 (46)	.27* (46)
ND	-.25 (46)	-.27 (39)	.36** (46)
SDMT: Written	.15 (46)	.23 (39)	-.33* (46)
Verbal	.22 (46)	.29 (39)	-.38** (46)
Rey Figure Test	.19 (46)	.13 (39)	-.09 (46)
COWAT	.23 (46)	.18 (39)	-.35** (46)
TMT: A	-.24 (46)	-.42** (39)	.46** (46)
TMT: B	-.33* (46)	-.4* (39)	.53** (46)
MSRT: Movement	-.21 (40)	-.4* (33)	.24 (40)
Choice	-.27 (46)	-.53** (33)	.36* (40)
MCRT: Movement	-.27 (40)	-.44* (33)	.31* (40)
Choice	-.08 (40)	-.29 (33)	.12 (40)

*p < 0.05, **p < 0.01 level (1-tailed); D=Dominant Hand, ND=Non-Dominant Hand; Number of patients in parenthesis

Outcome Measures

Glasgow Outcome Scale

All 47 patients were assessed using the extended GOS (GOSE) at 1 and 6-months post injury. Figure 3.2 illustrates 3 categories for the 5-point scale, obtained by collapsing the 8-point scale, and figure 3.3 represents the extended 8-point scale. As no patients had subsequently died from head injury or were in a persistent vegetative state, these categories were not used.

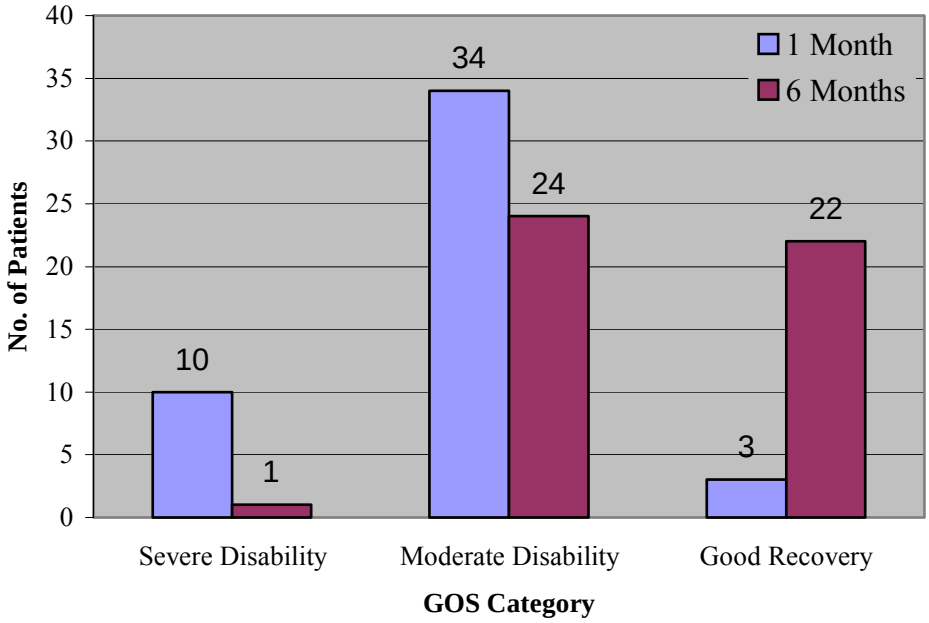


Figure 3.2. GOS scores at 1 and 6 months post injury

As can be seen in figure 3.2, there was an improvement in global outcome at 6 months in each GOS category. The majority of patients (72.3 %) were classed as being moderately disabled using the 5-point GOS at 1 month after injury. At 6 months, this figure had declined to 51.1 %. One patient (2.1 %) remained severely disabled at 6 months, a decrease from 10 patients (21.3 %) while those classed as

having a good recovery increased from 3 patients (6.4 %) at 1 month to 22 patients (46.8 %) at 6 months post injury.

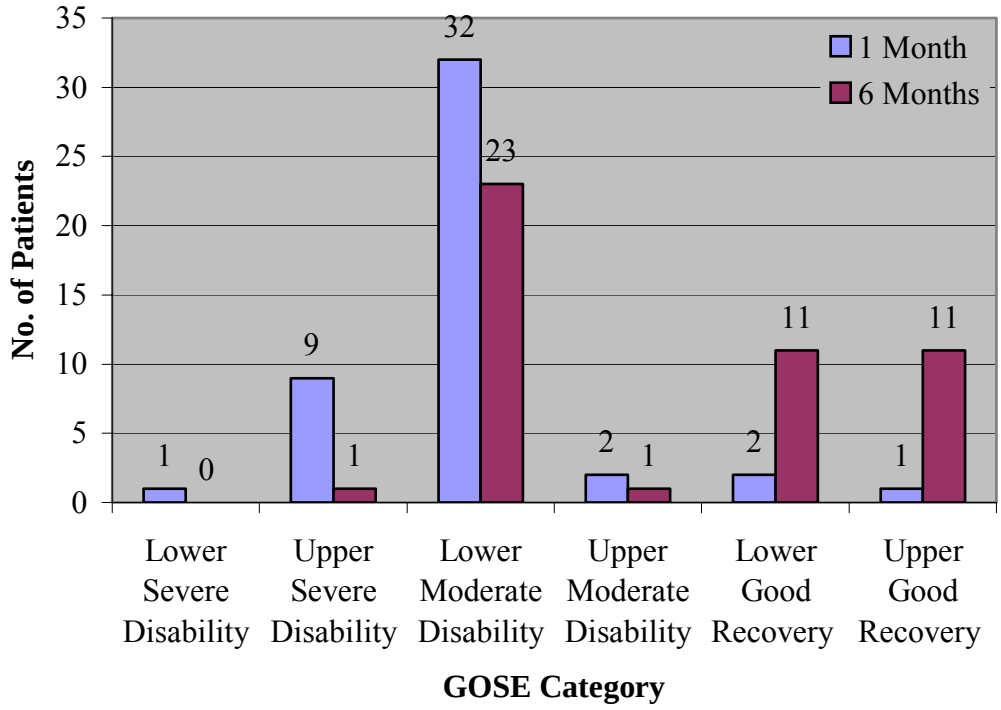


Figure 3.3. GOSE scores at 1 and 6 months post injury

Figure 3.3 displays 6 categories from the 8-point scale that mirrors the trends shown by the 5-point scale i.e. a decrease in severity of disability and an increase in favourable global outcome from 1 to 6 months post injury. Wilcoxon related samples tests show that improvement in global outcome is highly significant assessed by the 5-point scale ($z = -5.11$, $p < 0.001$) and 8-point scale ($z = -4.69$, $p < 0.001$) equally. The effect sizes for the GOS and GOSE between 1 and 6 months were found to be large at 1.14 and 1.09 respectively. A Spearman correlation performed on GOSE scores indicated that GOSE scores at 1 month were predictive of GOSE scores at 6 months ($r_s = 0.49$, $p < 0.01$).

Despite improvement over the 5-month period, both GOS and GOSE at 6 months identified that the majority of patients had some degree of disability.

Table 3.6. Correlation Between the GOSE and Clinical Indices of Severity

Index	N	GOSE 1 Month	GOSE 6 Months
GCS at A&E	47	$r_s = .14$	$r_s = .16$
24-Hour Worst GCS	40	$r_s = .34$*	$r_s = .26$*
PTA Estimate (Days)	47	$r_s = -.39$**	$r_s = -.23$*
PTA Grouping	47	$r_s = -.4$**	$r_s = -.17$

*p < 0.05 level, **p < 0.01 level (1-tailed)

One-tailed Spearman rank correlations highlighted significant correlations of GOSE with 24-Hour GCS and PTA (estimate and severity grouping). Higher scores on the GOSE equate to better outcome. With each clinical index of severity, the strength of association with GOSE scores weakened from 1 to 6 months post injury and with regards GOSE at 6 months and PTA severity grouping, there was no significant correlation (Table 3.6). GOSE at 6 months correlated significantly with age at injury ($r_s = -.33$, $p = .01$).

Table 3.7 illustrates the Spearman rank correlations between GOSE and neuropsychological test scores at 1 and 6 months. One patient had no 6-month scores thus, the corresponding 1-month scores were omitted from analyses. With regards 1-month test data, a trend existed whereby significant correlations with GOSE decreased in strength over the course of 5 months. Contrary to this trend, both immediate and delayed recall had a stronger relationship with GOSE at 6 months than at 1 month. Test scores at 1 month generally showed stronger associations with GOSE at 1 month than with GOSE at 6 months. Furthermore, test scores at 6 months were generally more strongly associated with GOSE at 6 months.

Table 3.7. Spearman Correlations Between GOSE and Test Scores

Task	N	GOSE 1 Month	GOSE 6 Months
1 Month			
Immediate Story Recall	46	$r_s = .19$	$r_s = .34$
Delayed Story Recall	46	$r_s = .14$	$r_s = .28$
Digit Span	46	$r_s = .26$	$r_s = .19$
BVRT	46	$r_s = -.45$**	$r_s = -.35$*
Pegboard: Dominant Hand	45	$r_s = -.33$*	$r_s = -.28$
Non-Dominant Hand	44	$r_s = -.36$**	$r_s = -.18$
6 Months			
Immediate Story Recall	46	$r_s = .17$	$r_s = .15$
Delayed Story Recall	46	$r_s = .09$	$r_s = .26$
Digit Span	46	$r_s = .24$	$r_s = .25$
BVRT	46	$r_s = -.26$	$r_s = -.5$**
Pegboard: Dominant Hand	46	$r_s = -.25$	$r_s = -.36$*
Non-Dominant Hand	46	$r_s = -.32$*	$r_s = -.26$
SDMT: Written Score	46	$r_s = .31$*	$r_s = .4$**
Verbal Score	46	$r_s = .35$*	$r_s = .44$**
TMT A	46	$r_s = -.3$*	$r_s = -.26$
TMT B	46	$r_s = -.27$	$r_s = -.3$*
Rey Figure: Delayed Recall	46	$r_s = .03$	$r_s = .22$
COWAT	46	$r_s = .08$	$r_s = .13$
MSRT: Movement Time	40	$r_s = .05$	$r_s = -.13$
Choice Time	40	$r_s = .03$	$r_s = .03$
MCRT: Movement Time	40	$r_s = .07$	$r_s = -.06$
Choice Time	40	$r_s = .12$	$r_s = -.27$

*p < 0.05 level, **p < 0.01 level (1-tailed)

Of the tests that were repeated at 6 months, there was a general decrease in strength of association with GOSE. Exceptions included delayed recall, BVRT error scores and Pegboard times using the dominant hand which showed a higher correlation with GOSE at 6 months. Tests that were exclusive to the 6-month assessment demonstrated significant associations with GOSE at 6 months on 2 occasions, TMT B and both subtests of the SDMT.

Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) was performed at each of the assessments and considered anxiety and depression separately, each with seven items rated from 0 to 3 (most severe) and scores totalled for each part. For both anxiety and depression, scores were categorised into 4 groups: a score of <7 is seen as normal, a score of 8–10 indicates mild symptoms, 11–14 indicates moderate symptoms and 15+ indicates severe symptoms.

Within the head injured group, 4 patients at 1 month and 3 patients at 6 months failed to complete and return the questionnaire. Thus, these patients were eliminated from analysis of HADS scores. Of the 41 remaining patients with completed HADS scores at 1 and 6 months, 35 % were recorded as having moderate or severe anxiety and 20 % with moderate or severe depression at 1 month. Although severe anxiety and depression levels decreased from 12 % to 7 % and 8 % to 2 % respectively, the number of patients with mild and moderate anxiety and depression levels increased. Mild and moderate anxiety levels increased from 27 % to 29 % and 22 % to 24 % respectively whereas mild and moderate depression levels increased from 12 % to 22 % and 10 % to 12 % respectively. Assessment at 1 month showed 39 % of patients were reported as having normal anxiety levels with 68 % recording no increased depression. At 6 months, normal anxiety levels remained the same at 68 % while normal levels of depression decreased to 63 %.

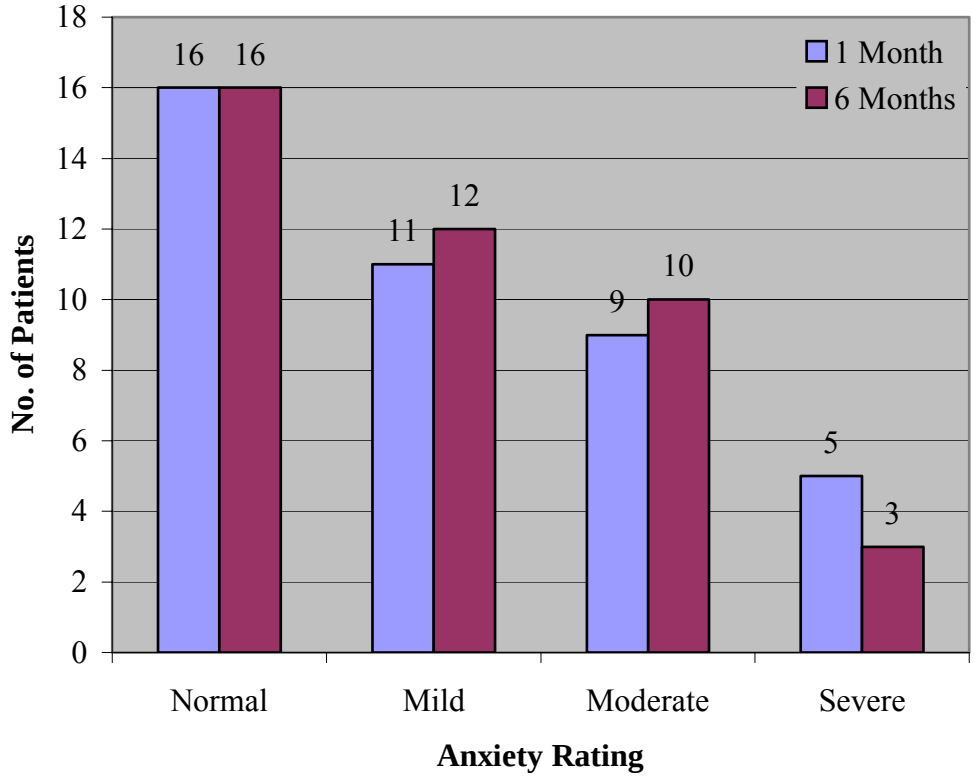


Figure 3.4. Anxiety scores at 1 and 6 months

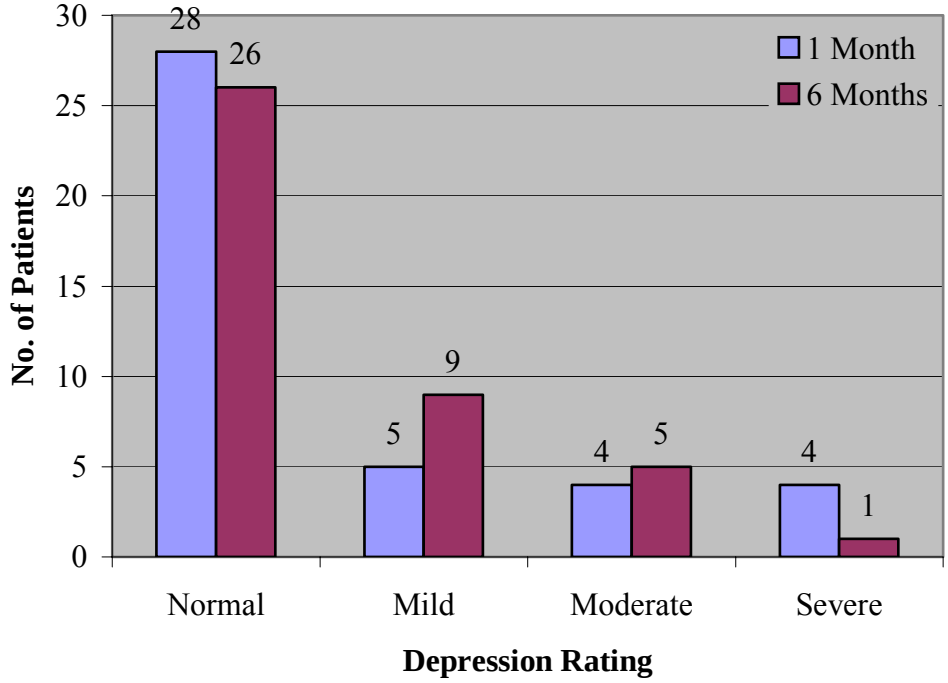


Figure 3.5. Depression scores at 1 and 6 months

Paired-sample t tests show that there were no significant differences between anxiety at 1 and 6 months, t (40) = -0.26, p = 0.8, or depression at 1 and 6 months, t (40) = 0.95, p = 0.35. Effect sizes were found to be small at 0.02 and 0.19 respectively. However, despite this and despite the mild classification of head injury, the majority of the patient group at both assessments after head injury had increased anxiety levels and a substantial number had experienced greater depression (figures 3.4 and 3.5).

Two-tailed Spearman correlations showed that anxiety and depression levels at time of assessment were highly correlated; anxiety and depression at 1 month post injury, $r_s = 0.8$, $p < 0.001$, and anxiety and depression at 6 months, $r_s = 0.82$, $p < 0.01$.

The mean anxiety scores at 1 and 6 months were 8.3 (SD 4.9) and 8.4 (SD 4.7) respectively while mean depression scores were 7 (SD 4.9) and 6.5 (SD 4.1) for 1 and 6 months respectively. Association of HADS scores at each GOSE assessment was carried out using 1-tailed non-parametric Spearman correlations (table 3.8).

Table 3.8. Spearman Correlation of HADS with GOSE

	GOSE 1 Month	GOSE 6 Months
N	41	41
HADS 1 Month		
Depression	$r_s = -.16$	$r_s = -.39$**
Anxiety	$r_s = -.22$	$r_s = -.55$**
HADS 6 Months		
Depression	$r_s = -.24$	$r_s = -.47$**
Anxiety	$r_s = -.34$*	$r_s = -.68$**

*p < 0.05, **p < 0.01 (1-tailed)

GOSE at 6 months significantly correlated with anxiety and depression at 1 and 6 months with anxiety having a stronger association and HADS having greater correlation generally at 6 months. GOSE at 1 month followed this trend although only 1 relationship was found to be significant, GOSE at 1 month with anxiety at 6 months. The HADS depression score showed a positive association with age at both 1 month, $r_s = 0.36$, $p = 0.02$, and 6 months, $r_s = 0.36$, $p = 0.02$ post injury.

Neurobehavioural Functioning Inventory

The Neurobehavioural functioning Inventory (NFI) has 6 categories each empirically demonstrated to be problematic for persons with a neurological disability: depression, somatic, memory/attention, communication, aggression, and motor. Both the patient and a relative completed the NFI for each assessment. The total number of NFI questionnaires completed by both patient and relative was 38; thus, 9 patients were eliminated from NFI results analysis.

Table 3.9. Spearman Correlations of Patient and Relative NFI

NFI 1 Month	r_s	p	NFI 6 Months	r_s	p
Depression	0.85	<0.001	Depression	0.78	<0.001
Somatic	0.83	<0.001	Somatic	0.90	<0.001
Memory/Attention	0.69	<0.001	Memory/Attention	0.65	<0.001
Communication	0.66	<0.001	Communication	0.61	<0.001
Aggression	0.71	<0.001	Aggression	0.70	<0.001
Motor	0.80	<0.001	Motor	0.84	<0.001

To identify potential problems with self-awareness, Spearman correlations were performed and showed highly significant associations ($p < 0.001$) between the NFI patient and relative scores for each of the 6 categories (table 3.9). This indicates that

both patients and relatives rated cognitive, emotional, physical and psychosocial states both before and after head injury similarly.

Paired-sample t tests were performed on the patient NFI data (table 3.10) and effect sizes calculated. Aggression and Motor scores were found to be significantly different at 1 and 6 months ($p < 0.05$) while Memory/Attention showed slight significance at $p = 0.07$. The accompanying effect sizes of these 3 aspects were found to be small.

Table 3.10. Paired-Sample Statistics of the NFI at 1 and 6 Months

	t	df	p	Effect Size
Depression	1	37	0.93	0.01
Somatic	0.82	37	0.42	0.1
Memory/Attention	-1.83	37	0.07	0.23
Communication	0.21	37	0.83	0.02
Aggression	-2.34	37	0.02	0.28
Motor	2.17	37	0.04	0.27

Spearman correlations were performed to determine association of GOSE score with NFI subscales (table 3.11). At 1 month, the patient depression subscale was associated with GOSE at 1 month and both patient and relative motor subscales were associated with GOSE at 1 month. GOSE at 6 months was more strongly associated with NFI with each patient and relative subscale providing significant correlations. There was a general trend between patient and relative NFI whereby associations with patient NFI subscales and GOSE tended to be stronger than relative NFI subscales.

Table 3.11. Spearman Correlations of NFI with Outcome Measured by GOSE

Patient NFI	GOSE 1 Month	GOSE 6 Months	Relative NFI	GOSE 1 Month	GOSE 6 Months
1 Month:					
Depression	-.27*	/	Depression	-.08	/
Somatic	-.14	/	Somatic	-.09	/
Memory/Attention	-.13	/	Memory/Attention	-.25	/
Communication	-.21	/	Communication	-.21	/
Aggression	-.22	/	Aggression	-.17	/
Motor	-.41**	/	Motor	-.33*	/
6 Months:					
Depression	/	-.58**	Depression	/	-.57**
Somatic	/	-.65**	Somatic	/	-.60**
Memory/Attention	/	-.62**	Memory/Attention	/	-.50**
Communication	/	-.55**	Communication	/	-.51**
Aggression	/	-.50**	Aggression	/	-.32*
Motor	/	-.63**	Motor	/	-.63**

*p < 0.05 level, **p < 0.01 level (1-tailed)

Return to Work/Social & Leisure Activities

Employment status was ascertained at each assessment. Of the group of 47 patients, the majority (42) were in full or part-time employment or further/higher education pre-injury with 4 unemployed and 1 retired (figure 3.6). Only 4 % of patients returned to work 1 month after injury while 94 % were too incapacitated to return to working life. At 6 months after injury, 43 % had returned to work although the majority of patients (55 %) had still not returned to work.

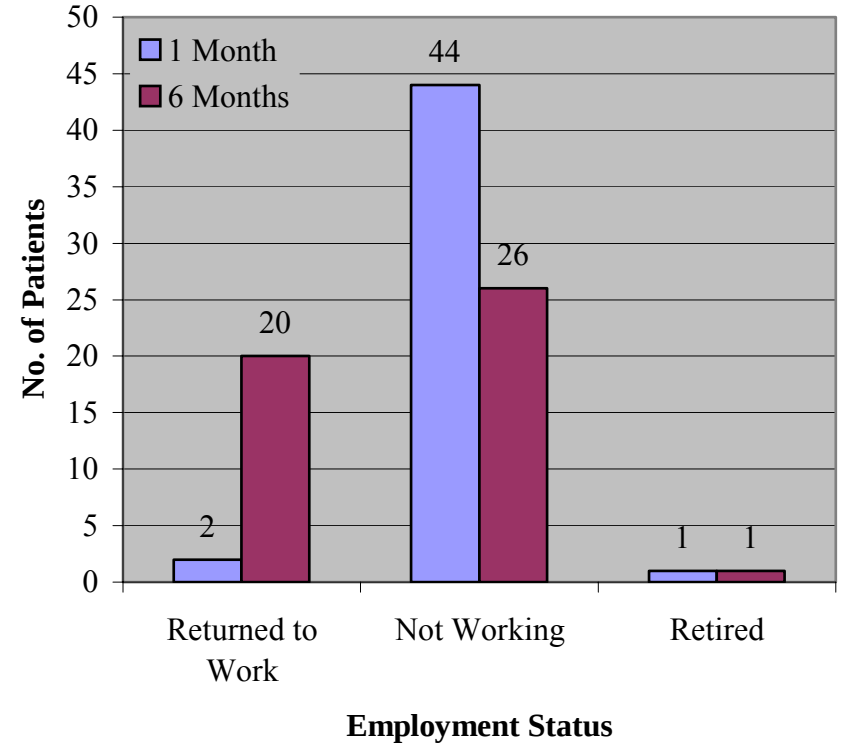


Figure 3.6. Employment status 1 and 6 months post injury

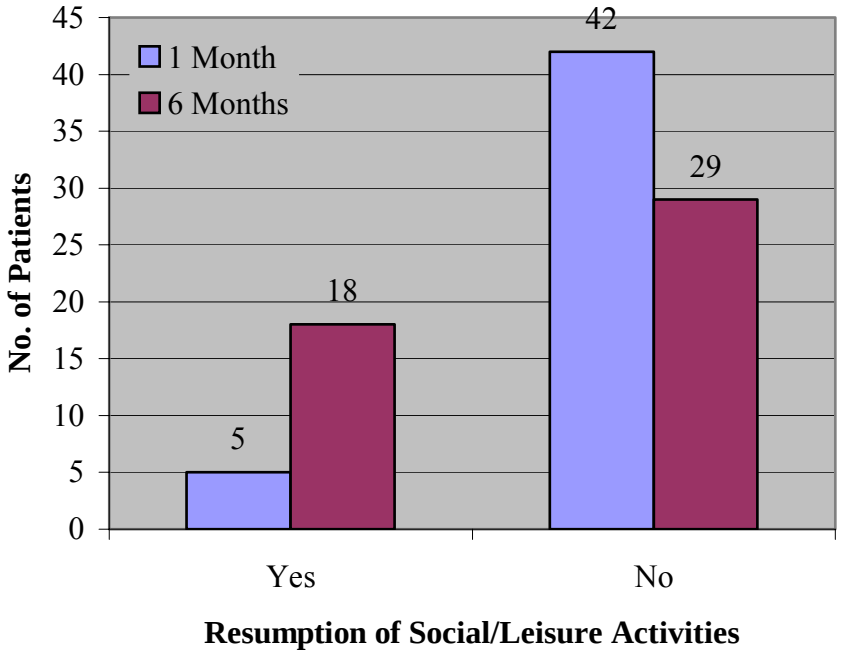


Figure 3.7. Status of social and leisure activities

All 47 patients had participated in some form of regular social and leisure activity pre-injury. At 1 month post injury, 11% reported resumption of social and leisure activities with 89% reporting that they were not as active in these areas as they once were. At 6 months, the number of patients resuming such activities rose to 38% although the majority of 62% still felt unable to return to normal levels (figure 3.7).

Table 3.12. Injury Severity Measures and Return to Work and Social Activities

	A&E GCS (n = 47)	24 Hr GCS (n = 40)	PTA (n = 47)
Return at 1 Month			
Work	.21	.16	-.25*
Social Activities	.10	-.03	-.05
Return at 6 Months			
Work	.17	.21	-.19
Social Activities	.13	.04	-.12

*p < 0.05 level (1-tailed)

Spearman correlations revealed one significant correlation, PTA as a measure of injury severity with return to work at 1 month. There was a general trend whereby correlations with return to work were stronger than correlations with return to social activities (table 3.12).

Neuropsychological Test Scores

For the Grooved Pegboard task at 1 month, 1 patient was unable to perform the task using either hand and one patient was unable to utilise their non-dominant hand. The apparatus used for the Reaction Time Tests was inoperative on 6 occasions resulting in failure to obtain reaction time data for 6 patients and 7 controls. One patient had no 6-month test scores and was thus omitted from analyses. A summary of the test scores at 1, 3 and 6 months is given in table 3.13.

Table 3.13. Summary of Cognitive Scores at 1, 3 and 6 Months Post injury

	N	Min	Max	Mean	SD
1 Month					
Immediate Story Recall:					
No. Correct	46	1	13	6.1	2.8
Delayed Story Recall:					
No. Correct	46	0	11	4.8	2.7
Digit Span:					
Total Correct	46	8	24	14.4	3.6
BVRT:					
Total Errors	46	0	14	5.2	2.9
Pegboard: (secs)					
Dominant Hand	45	59	177	87.5	24.2
Non-Dominant Hand	44	7	234	91.9	33.5
3 Months					
Immediate Story Recall:					
No. Correct	46	1	16	7.3	3.6
Delayed Story Recall:					
No. Correct	46	1	15	6.5	3.4
Digit Span:					
Total Correct	46	9	23	14.4	3.5
BVRT:					
Total Errors	46	0	15	5.8	3.4
Pegboard: (secs)					
Dominant Hand	46	58	154	81.2	20.8
Non-Dominant Hand	46	60	159	87.7	23.7
6 Months					
Immediate Story Recall:					
No. Correct	46	3	16	8.3	3.3
Delayed Story Recall:					
No. Correct	46	0	16	7.2	3.4
Digit Span:					
Total Correct	46	7	22	15.4	3.4
BVRT:					
Total Errors	46	0	14	4.4	3.0
Pegboard: (secs)					
Dominant Hand	46	53	159	78.0	20.4
Non-Dominant Hand	46	14	160	82.9	23.8
SDMT:					
Written Score	46	16	73	41.3	10.7
Verbal Score	46	18	78	48.7	12.7
Rey Figure:					
3 min Delay	46	6	36	21.3	7.9
COWAT:					
Total Letter Score	46	10	80	32.6	12.2
TMT A:					
Time (secs)	46	18	92	40.2	15.3
TMT B:					
Time (secs)	46	39	140	83.5	27.6
MSRT:					
Movement Time (secs)	40	145	333	250	45.8
Choice Time (secs)	40	211	353	275	32.9
MCRT:					
Movement Time (secs)	40	145	323	259	47.7
Choice Time (secs)	40	214	358	290	27.2

Table 3.14. Effect Sizes for Comparisons Between Patient Scores

Task	Effect Size	t	p
Between 1 and 3 Months			
Immediate Story Recall	0.37	2.12	<0.05
Delayed Story Recall	0.52	3.1	<0.01
Digit Span	0.01	0.06	0.95
BVRT	0.22	1.53	0.13
Pegboard: Dominant Hand	0.32	3.1	<0.01
Pegboard: Non-Dominant Hand	0.18	1.23	0.22
Between 1 and 6 Months			
Immediate Story Recall	0.74	4.64	<0.001
Delayed Story Recall	0.76	5.11	<0.001
Digit Span	0.26	2.63	<0.01
BVRT	0.26	1.71	0.09
Pegboard: Dominant Hand	0.22	5.09	<0.001
Pegboard: Non-Dominant Hand	0.16	3.43	<0.001

Effect sizes were calculated for each of the tests performed at 1, 3 and 6 months (table 3.14) to give an indication of the size of the effect that change over time had on test scores that allows comparison between different measures. Effect sizes were found to be small except for delayed story recall between 1 and 3 months and immediate and delayed story recall between 1 and 6 months where the effect sizes were moderate, 0.52, 0.74 and 0.76 respectively. Figure 3.8 illustrates mean patient story recall scores. Within the patient group, immediate and delayed recall improved significantly between 1, 3 and 6 months with more information being recalled. Independent sample t tests were performed between patient and control scores for each test and at each assessment interval and corresponding effect sizes calculated. For immediate and delayed recall at 1 month, patient and control scores were significantly different, $t(61) = 2.64$, $p < 0.01$ and $t(61) = 3.78$, $p < 0.001$ respectively. Respective effect sizes were large, 0.83 and 1.19, indicating that brain injury had a substantial consequence for story recall.

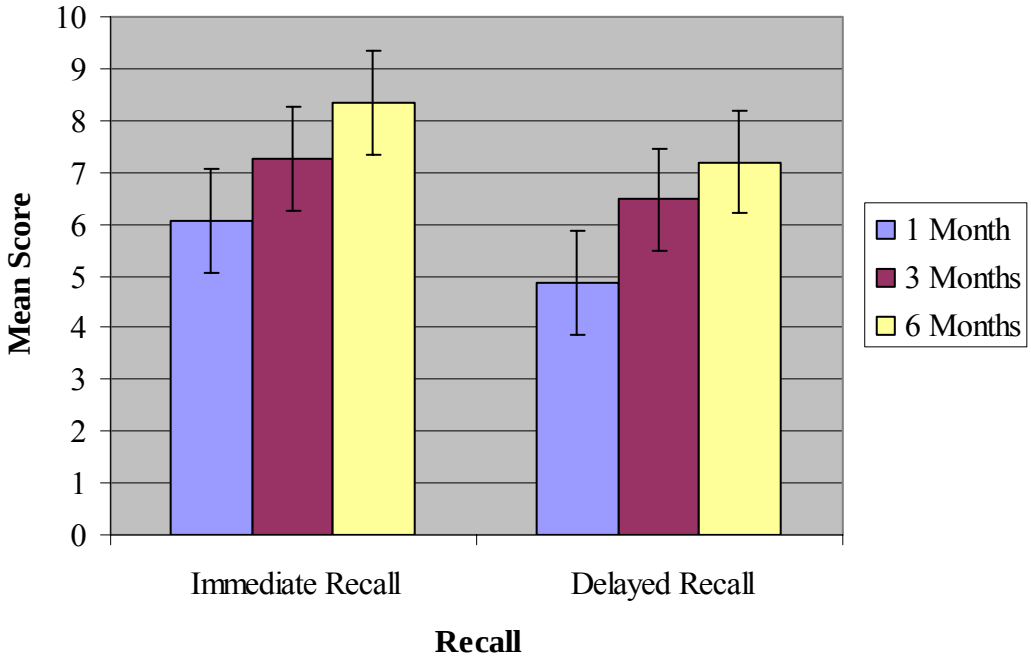


Figure 3.8. Patient Story Recall scores at 1, 3 and 6 months

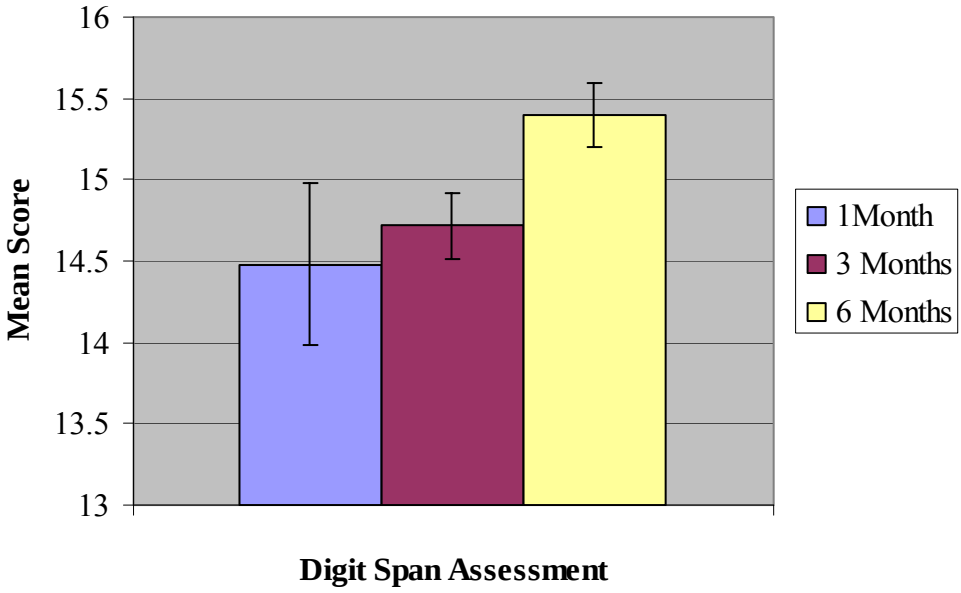


Figure 3.9. Patient Digit Span scores at 1, 3 and 6 months

Digit span was seen to improve over the duration of the 3 assessments with scores increasing from a mean of 14.48 at 1 month to 15.37 at 6 months (figure 3.9). There were no significant differences in digit span between 1 and 3 months. Although there was significance between 1 and 6 months, the effect size was small at 0.26 indicating that there was relatively little change over time in Digit Span. When comparing patient and control groups, digit span scores differed at each assessment with $t(61) = 2.77$, $p < 0.01$ at 1 month, $t(61) = 3.92$, $p < 0.001$ at 3 months and $t(61) = 3.12$, $p < 0.01$ at 6 months. Respective effect sizes were 0.74, 1.10 and 0.90; indicative that brain injury had a substantial effect on digit span. For BVRT scores within the patient group there were no significant differences between assessments and effect sizes were small at 0.22 and 0.26. However, the scores indicate a trend in the predicted direction from 1 to 6 months post injury with error scores decreasing (figure 3.10). In comparison to the control group, scores at the 3 month assessment differed, $t(61) = 2.36$, $p < 0.05$ with a corresponding moderate effect size of 0.70.

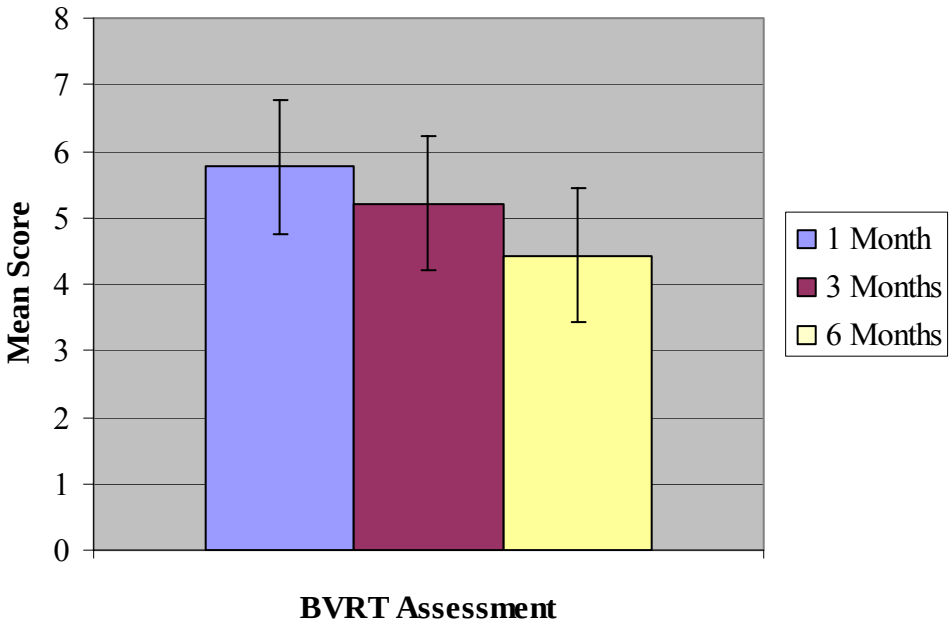


Figure 3.10. Patient BVRT scores at 1, 3 and 6 months

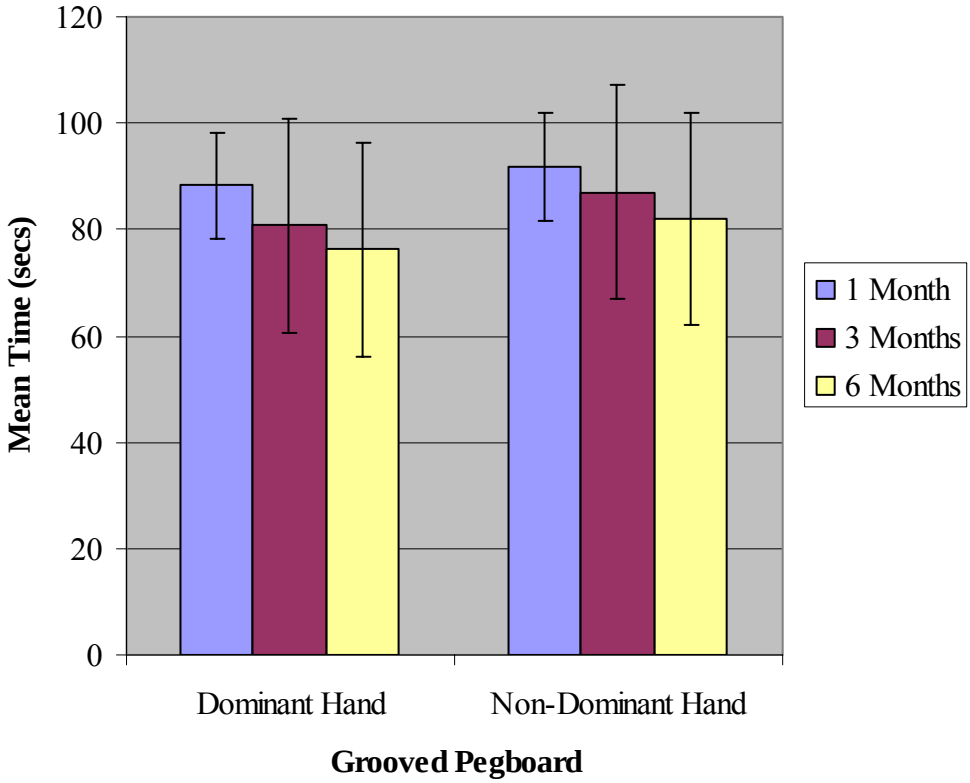


Figure 3.11. Comparative Patient Grooved Pegboard scores

Results from the Grooved Pegboard task were shown to improve over time with a reduction in mean times for both the dominant and non-dominant hand (figure 3.11). Within the patient group, differences were significant for the dominant hand between 1 and 3 months and for the dominant and non-dominant hand between 1 and 6 months (table 3.14). When compared to the control group, significant differences were achieved for the dominant hand at each assessment with $t(60) = 2.51$, $p = < 0.05$ at 1 month; $t(61) = 2.24$, $p < 0.05$ at 3 months and $t(61) = 2.01$, $p = < 0.05$ at 6 months. Effect sizes were 0.77, 0.70 and 0.63 respectively.

Regarding cognitive tests administered solely at 6 months i.e. the Symbol Digit Modalities Test, the COWAT, Trail Making A and B, the Rey Figure test and the

Reaction Time tests, evidence of impairment due to head injury was apparent due to significant associations with indices of severity, especially post traumatic amnesia (table 3.5). With regard to comparisons with the control group on the cognitive tests administered soley at 6 months, the Trail Making Test B performance between the groups was significantly different, $t(61) = 2.25$, $p < 0.05$ with a medium effect size of 0.67.

Correlational analysis was performed on NART error scores and cognitive test data. As expected, the NART error score correlated significantly with a number of cognitive tests at each assessment (table 3.15). However, there were no significant differences in NART error scores between patient and control groups, $t(61) = 2.1$, $p = 0.06$. Significant age correlations were found with the BVRT error score ($r = 0.26$, $p < 0.05$ at 3 months and $r = 0.37$, $p < 0.01$ at 6 months), the SDMT Written score ($r = -0.36$, $p = 0.01$) and the Rey Figure test ($r = -0.41$, $p < 0.01$). However, there were no age differences between groups, $t(61) = 1.8$, $p = 0.09$.

Table 3.15. Correlations of NART Error Scores with Cognitive Tests

Assessment	Test	r	p
1 Month	Digit Span	-0.43	<0.01
	BVRT	0.37	<0.05
3 Months	Immediate Story Recall	-0.54	<0.001
	Delayed Story Recall	-0.47	<0.01
	BVRT	0.51	<0.01
6 Months	Immediate Story Recall	-0.39	<0.05
	Delayed Story Recall	-0.46	<0.01
	Digit Span	0.44	<0.01
	BVRT	-0.34	<0.05
	SDMT Written	-0.32	<0.05
	SDMT Verbal	-0.39	<0.05
	Rey Figure	-0.42	<0.05
	COWAT	-0.62	<0.001

Discussion

Clinical Indices of Severity

The Glasgow Coma Scale and Post Traumatic Amnesia are highly regarded as predictors of outcome after head injury and are widely used. The current study found PTA to be associated with the GOSE at 1 month with weaker correlation at 6 months post injury with the same trend repeated with the 24 hour worst GCS. The weaker correlations at 6 months can be explained by recovery from injury over a period of time. These findings indicate that longer duration of PTA and severity of injury as measured by the GCS leads to poorer outcome measured by the GOSE. This is consistent with previous research on severe head injured patients that reported strong association with outcome as measured by the GOS and duration of PTA and GCS score on admission to hospital (Bishara et al., 1992).

Correlations of test scores with GCS at A & E and worst GCS at 24 hours displayed a trend towards a relationship between severity of injury and test performance with significant correlations for Grooved Pegboard at 1 month and Trail Making tests and Reaction Times at 6 months. In this study, PTA proved to be a stronger indicator of injury severity with more significant associations with test scores than GCS. The direction of association was consistent with poorer outcome in relation to increased duration of PTA in all but two correlations, immediate story recall and digit span at 1 month. PTA has been shown to be a superior predictor of outcome compared to GCS in mild and moderate head injury. Research using a patient group with a GCS score of 13 or more, assessed at 5-6 months post injury, found better association between PTA and indices of outcome compared to GCS (McCullagh et al., 2001) and

outcome, as measured by the GOS and a more detailed scale, in patients with GCS 9-14 was determined by duration of PTA and not GCS score (Van der Naalt et al., 1999).

Outcome Measures

Neurobehavioural Functioning Inventory

For each of the six categories on the NFI, there was significant agreement between patient and relative scores indicating that in this patient group, self-awareness was not affected by head injury. General agreement between patient and relative scores has also been reported in a study of 301 head injured patients (Seel, Kreutzer & Sander, 1997) and NFI validity has been ascertained by examining the relationships between the six NFI categories and appropriate neuropsychological tests (Kreutzer et al., 1996). In the current study, changes in functioning between 1 and 6 months were significant for aggression and motor scores and memory/attention were slightly significant. Changes in such varied modalities are indicative of brain injury with a diffuse nature whereby damage is not restricted to isolated areas of the brain.

Hospital Anxiety and Depression Scale

The majority of patients were recorded as having mild, moderate or severe anxiety levels at both assessments. Most patients had no depressive symptoms but depression was recorded by a number of patients large enough as not to be ignored. GCS scores did not show a significant relationship with the HADS although the

GOSE 1 month score was associated with anxiety at 6 months and the GOSE 6 month score was associated with anxiety and depression score at both assessments with stronger correlations for anxiety.

Depression and anxiety are frequently reported after head injury with assessment made using various methods such as the Beck Depression Inventory (BDI), the Beck Anxiety Index (BAI), the NFI, the HADS, the Diagnostic Statistical Manual for Mental Disorders 4th edition (DSM-IV) and the Minnesota Multiphasic Personality Inventory–2. The NFI has been used to identify depressive symptomatology in line with the DSM-IV criteria in a population of 722 head injured patients with fatigue, frustration and poor concentration being the most common symptoms reported (Kreutzer, Seel & Gourley, 2001). Seel and colleagues (2003) assessed a patient group of 666 head injured patients 10-126 months post injury. NFI responses indicated that 27% of patients met DSM-IV criteria for depression with feelings of hopelessness and worthlessness and anhedonia being three symptoms to best distinguish depressed from non-depressed patients (Seel et al., 2003).

Depressive symptoms using the HADS have been previously identified in head injured patients in comparison to healthy and orthopaedic controls (Watt & O'Carroll, 1999). In the current study, differences in anxiety and depression levels between 1 and 6 months post injury did not reach statistical significance. Nevertheless, the majority of the patient group displayed anxious symptoms and a substantial number displayed depressive symptoms. The current findings from the raw scores of the depression scale in the NFI produced small effect sizes between assessments indicating that there was no change in mood regarding depression as

measured by the NFI. Depressive symptoms have been reported in mild and moderate head injured patients assessed at 2 months post injury (Goldstein & Levin, 2001) and in mild head injured patients at 3 months (Levin et al., 2005). The lack of actual significant findings in the current study may be due to the current patient group having milder head injury than the aforementioned studies and/or the assessment procedure used to identify depression. However, as mentioned previously, about a third of patients at both assessments were recorded as having depressive symptoms and the number of normal patients decreased over the 5 month period. Thus, depression was an issue in this patient group. There is evidence to suggest that neuropathological sequelae are a contributory factor to the development of depression after head injury. Patients with head injury assessed 1 year post injury were found to have a higher frequency of major depressive disorder than a patient group who had endured comparable levels of injury but without brain injury (Jorge et al., 2004).

Head-injured patients often report "free-floating" generalised anxiety which is accompanied with fearfulness, worry and tension (Lewis & Rosenberg, 1990). In the present study there was no significant difference in anxiety levels as measured by the HADS between 1 and 6 months post injury. However, the majority of the patient group had some form of anxiety at both assessments; over half the patients had anxiety symptoms at 1 and 6 months. Therefore, it is appropriate to state that anxiety was a problem in the current patient group. Increased anxiety is commonly reported in head injured populations (Jorge & Robinson, 2003; Ponsford et al., 2002; Goldstein & Levin, 2001; Wallace & Bogner, 2000) and symptoms have been reported to have increased one year post injury (Van der Naalt et al., 1999a).

However, others have detected no increased anxiety after head injury (McAllister et al 2001a; Watt & O'Carroll, 1999). One possibility to explain such discrepancies in the literature may be the technique used for assessment of anxiety.

Glasgow Outcome Scale

There was significant improvement in the patient group between 1 and 6 months post injury as measured by the both the GOS and extended GOS. There were also modest associations between GOSE with PTA and 24 hour worst GCS indicating that poorer outcome resulted from greater severity of injury. Stronger associations were found with PTA which corresponds with the findings that PTA was a better predictor of outcome as determined by correlation with neuropsychological test scores. The correlations were weaker at 6 months suggesting improvement in the patient group after time. PTA as a measure of injury severity has also been found to be superior to GCS for assessing outcome using the GOSE in a group of 135 head injured patients (Wilson, Pettigrew & Teasdale, 2000).

With the exception of the aggression subscale at 1 month, both patient and relative NFI subscales at 1 and 6 months were correlated with GOSE at 6 months. GOSE at 6 months produced more associations and was more strongly associated with NFI than GOSE at 1 month. A general trend was evident between patient and relative NFI whereby associations with patient NFI subscales and GOSE tended to be stronger than relative NFI subscales. These results are in contrast to a previous study of head injured patients 6 months post injury that also reported correlations of NFI

subscales with GOSE but stronger correlations were found with relative NFI (Wilson, Pettigrew & Teasdale, 2000).

A possible explanation for this apparent discrepancy involves severity of injury. In the latter study where worst recorded GCS score was used, 36 % of the patients had severe injury, 21 % had moderate injury and 44 % had mild injury. Of the worst recorded GCS scores available for 40 patients in the current study, 25 % had severe injury, 13 % had moderate injury and 62 % had mild injury. The current finding whereby patient neurobehavioural reports were more strongly associated with outcome may be indicative that this mildly head injured population had less cognitive deficit and increased self awareness and could provide more accurate neurobehavioural assessment. It has been found that moderate to severe head injured patients who reported fewer deficits when compared to assessment by a relative, also tended to report fewer symptoms of depression and anxiety (Wallace & Bogner, 2000). In severely head injured patients assessed up to 2 years post injury, Port and colleagues (2002) found no significant differences between overall scores from the Awareness of Deficits Questionnaire although relatives commonly reported more severe deficits than the subject (Port, Willmott & Charlton, 2002). A lack of self-awareness of deficits due to head injury has rehabilitation and psychological adjustment implications. An in-depth analysis of self-awareness was outwith the realms of the current study.

Clifton and associates (1993) investigated the relationship between neuropsychological test data and the GOS in severe and moderate head injured patients where GOS at 3 and 6 months post injury was compared with 19

neuropsychological tests. Of these 19 tests the COWAT, Grooved Pegboard, delayed recall of the Rey Figure and Trail Making Test B were most strongly related to outcome scores. Analysis of composite scores of these 4 tests resulted in variable means between GOS classifications which were accompanied with wide standard deviations indicating performance variability across the GOS classifications (Clifton et al., 1993). In the current study, correlations of the COWAT and delayed recall of the Rey Figure with GOSE did not reach significance. Trail Making Test B was significantly associated with GOSE at 6 months while part A was associated with GOSE at 1 month. With regards the Grooved Pegboard at 1 month, using both the dominant and non-dominant hand, there were associations with GOSE 1 month and using the non-dominant hand at 6 months. The Grooved Pegboard using the dominant hand at 6 months was the only association with GOSE at 6 months.

With regards the remaining cognitive tests, GOSE at 1 month was found to be significantly associated with the Benton Visual Retention Test at 1 month and the Symbol Digit Modalities Test and Trail Making Test A at 6 months. GOSE at 6 months was significantly associated with immediate recall at 1 month, the Benton Visual Retention Test at both 1 and 6 months and the Symbol Digit Modalities Test and Trail Making Test B at 6 months. Previous research with GOSE scores taken at mean 7.4 months post injury has also found associations with the Symbol Digit Modalities Test, The Trail Making Test B and Grooved Pegboard although in contrast with the current findings, the latter study also found associations with the COWAT and immediate and delayed recall (Wilson, Pettigrew & Teasdale, 2000).

Despite findings in the literature regarding association of neuropsychological measures and outcome as measured by the GOS/GOSE, the same research has also documented sizeable overlap in scores between the classification groups (Levin et al., 1979; Clifton et al., 1993; Wilson, Pettigrew & Teasdale, 2000). It was not feasible to investigate neuropsychological test scores in the individual GOSE classifications due to limited numbers of patients in each category. Combination of GOS scores and test data would appear to provide an opportunity to develop a more detailed assessment of outcome but such overlap of test scores between GOS classifications make this difficult. Neuropsychological measures of language and attention collected at 1 month post injury from mild to severe head injured patients have suggested that data regarding the actual test completion rates of patients rather than raw scores is an improved method for the prediction of outcome scores (Pastorek, Hannay & Contant, 2004). Batteries of neuropsychological tests have found variability in outcome 5 years after head injury ranging from no impairment to severe impairment (Millis et al., 2001) and as yet, there are no cognitive tests that have been found to correlate strongly enough with GOS to enable a simple combination (Wilson, 2001). Moreover, GOS assesses disability and handicap whereas neuropsychological testing is aimed to assess specific functions of specific parts of the brain. Thus, although both methods assess outcome, they do so for quite different aspects of head injury (Wilson, 2001).

Return to Work/Social Activities

The premorbid employment status showed that 89 % of the patient group were in employment or further/higher education. Postmorbid status at 1 month revealed that

only 4 % were able to return to work while after 6 months, this figure rose to 43 %. The same pattern was displayed for social and leisure activity; at 1 month post injury, 11 % of the patient group had resumed social and leisure pursuits while this figure increased at 6 months to 38 %. It is interesting to note that although this was predominantly a mild head injured population as measured by the GCS at A & E and 24 hour worst GCS, the majority of patients were too incapacitated after a period of 6 months to return to normal levels of occupational status and social pursuits. Kersel and colleagues (2001) found levels of return to work at 6 months after a severe head injury to be low with 13 % of the patient group being able to return to the work environment and 30 % in paid employment after 1 year post injury. Resumption of social and leisure activities was higher with 62 % of patients being socially active at 1 month and 70 % after 1 year (Kersel et al., 2001).

Correlations with indices of injury severity revealed no significant associations except that of return to work at 1 month with PTA. Failure to find associations with the GCS was found in a previous study by Felmingham and colleagues where GCS contribution to return to work prediction was analysed against age and employment status. When GCS was combined with age at injury, it became significant as a predictor of return to work (Felmingham, Baguley & Crooks, 2001). The latter study used lowest GCS score within 24 hours as did the current study which also included GCS at A&E. A similarity between the two studies which may explain the lack of association between GCS score and return to work is that both had a limited range of severity within the patient group; Felmingham and colleagues had a limited range of patients with mild head injuries and the current study had a limited range of severe head injured patients. However, research investigating GCS and return to work has

also been performed specifically on mild head injured patients with a GCS score of 13 or greater. Patients were assessed at 5-6 months post injury and return to work was not found to be associated with GCS score (McCullagh et al., 2001).

Neuropsychological Testing

Head Injury and Memory

In the current study, memory was specifically investigated using immediate and delayed story recall, the Benton Visual Retention Test (BVRT) and delayed recall of the Rey Figure. Performance on immediate and delayed story recall significantly improved between 1 and 3 months and between 1 and 6 months indicating that neuropsychological recovery with regard to verbal memory had improved in this time period. It has been shown that recovery from moderate to severe head injury may continue in some patients up to 5 months post injury; patients assessed at 1 and 5 years after injury were found to have significant improvement in cognitive abilities including verbal memory (Millis et al., 2001). Deficits in immediate and delayed recall have been illustrated in severe head injury (Nissley & Schmitter-Edgecombe, 2002; Schmitter-Edgecombe & Woo, 2004) and verbal recall has been implicated with improvement after 6 weeks in very mild head injury (Voller et al., 1999).

Visuo-spatial memory was measured by delayed recall of the Rey Figure at 6 months. Poor performance on the Rey Figure Test has been previously shown in severe head injury (Serra-Grabulosa et al., 2005; Zec et al., 2001) and has been distinguished in type of injury where patients with brain stem and callosal lesions but

not frontotemporal lesions displayed poorer recall in comparison to controls (Wilson et al., 1995). The BVRT, another measure of visuo-spatial memory, was used at 1, 3 and 6 months post injury. Scores between patients and controls were significantly different at 3 months but within the patient group, scores were not found to be significantly different between assessments. However, the scores indicated a trend in the predicted direction from 1 to 6 months post injury whereby there was a decrease in error scores. Reduction of BVRT errors at 6 months implies a certain degree of improvement within the patient group.

Head Injury and Attention

Digit span is a measure of attention and short-term memory and was measured at 1, 3 and 6 months post injury. Although the effect size was small, there was a significant increase in performance from 1 to 6 months and there were significant differences in scores between patients and controls at 1, 3 and 6 months indicating that the patient group had decreased attention and short-term memory following head injury. Digit span performance in severe head injured patients has been previously found to be similar to that of controls (Nissley & Schmitter-Edgecombe, 2002; Heilbronner & Henry, 1991) although severely head injured subjects performed worse on the backwards digit span when compared to controls (Schmitter-Edgecombe & Woo, 2004). Also, it would appear that type of injury is not an issue with regard to digit span score; severe diffuse and focal injuries have both been involved in reduced digit span (Wilson et al., 1995).

Head Injury and Verbal Fluency

Verbal fluency was measured at 6 months using the COWAT. COWAT performance in relation to control groups has been shown to be inferior in moderate and severely injured patients (Mathias et al., 2004; Schmitter-Edgecombe & Woo, 2004) and, although not significant, a trend has been shown in very mild head injured patients (Voller et al., 1999). Poor performance has also been found regardless of injury type; patients with either severe diffuse or severe focal injury both achieved inferior scores on the COWAT (Wilson et al., 1995) and assessment of moderate and severe head injured patients has found significant improvement in verbal fluency from 1 to 5 years post injury (Millis et al., 2001).

Head Injury, Visual-Motor Integration and Mental Processing Speed

Visual-motor integration and mental processing speed were measured at 1, 3 and 6 months using the Grooved Pegboard and at 6 months only using the Symbol Digit Modalities Test (SDMT) and the Trail Making Test (TMT) parts A and B. Severely head injured patients have been reported to give poorer performances on the TMT, the Digit Symbol subtest of the WAIS-R, the Symbol Digit Modalities test and the Grooved Pegboard (Schmitter-Edgecombe & Woo, 2004; Nissley & Schmitter-Edgecombe, 2002; Heilbronner & Henry, 1991; Wilson et al., 1995; Clifton, et al., 1993). Performance on the Grooved Pegboard using both dominant and non-dominant hand had significantly improved between 1 and 6 months reflected by reduced completion times. Comparison with the control group illustrated significant differences in Grooved Pegboard scores using the dominant hand at 1, 3 and 6 months post injury. Comparison of TMT scores between patient and control groups

revealed a significant difference in TMT B scores. Although some have reported no differences in TMT score between controls and mild head injured patients (Dikmen, Machamer & Temkin, 2001), others have recorded impairment in severe head injury (Felmingham, Baguley & Green, 2004; Nissley & Schmitter-Edgecome, 2002).

Global outcome after head injury, as discussed earlier, has been related to reduced motor speed on the Grooved Pegboard (Clifton, et al., 1993; Wilson, Pettigrew & Teasdale, 2000) and the SDMT and TMT B (Wilson, Pettigrew & Teasdale, 2000). As mentioned earlier, the current study found completion times using both hands for the Grooved Pegboard at 1 month to be associated with outcome at 1 month and using the dominant hand at 6 months with outcome at 6 months. Outcome was also associated with the SDMT and TMT part A at 1 month and TMT part B at 6 months.

Thus, the data implies that impairment of visual-motor integration and mental processing speed within the patient group occurred sufficiently to be detected by improvement on the Grooved Pegboard and to have produced associations of SDMT, TMT and Grooved Pegboard with clinical indices of severity. Therefore, such tests in predominantly mild head injury would complement the GOSE enabling a greater scope of outcome in such patient groups.

Head Injury and Processing Speed

Processing speed was measured at 6 months using Simple and Choice Reaction Time tests. There were no significant differences between the scores from the patient and control groups. However, as with other cognitive tasks, reaction times produced

some associations with severity of injury as measured by GCS and PTA. Thus, indicating impairment due to head injury. Previous research has reported patients with mild, moderate or severe head injury to have reduced reaction times when compared to controls (Salmond et al., 2005; Mathias, Beall & Bigler, 2004; Mathias et al., 2004; Sarno et al., 2003; Nissley & Schmitter-Edgecome, 2002) and reduced reaction time has also been reported in very mild head injured patients (Voller et al., 1999).

Summary

In summary, the neuropsychological test findings imply that head injury in the patient group, although mild, was sufficient to produce cognitive deficits within the patient group itself as identified by comparison of within-group results, comparison of results at each assessment with the orthopaedic control group and by association with clinical indices of injury severity. Expected relationships were found between injury severity, global outcome and cognitive tests whereby poorer cognitive performance was associated with greater injury severity and poorer outcome. With regards longitudinal cognitive changes, association of injury severity with cognitive scores was seen to weaken over time and this trend was also found with GOSE although only moderately so. Global outcome was seen to significantly improve over the 5-month period with the 1-month GOSE score predicting the 6-month GOSE score. Cognitive assessment indicated an improvement in abilities for tasks performed at 1, 3 and 6 months post injury. There was no significant change in emotional and behavioural states as measured by the NFI and HADS. However, with a sizeable number of patients having reported anxiety and depressive symptoms, it

can be stated that neurobehavioural problems were reflected in this patient group. The evidence that the majority of patients were unable to resume normal levels of social activity and employment status supports these findings.

The demographics of the current study were similar to those of other studies. The patient group was typical in age range, cause of injury and injury severity i.e. patients were predominantly mildly head injured as assessed by the GCS as is usual for patients who report to A&E departments with head injury (Kay & Teasdale, 2001). However, despite this mild classification, the patient group were a predominantly complicated mild head injury group typical of neurosurgical samples. Also, despite displaying improvement between cognitive assessments, impairment was evident due to comparisons of cognitive scores with the control group, associations with injury severity and a high proportion of patients exhibiting depressive and anxious symptomology.

CHAPTER 4: NEUROANATOMICAL VOLUMETRIC ANALYSIS

Overview of Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has made possible the identification and study of brain structures such as the amygdala (Sachdev et al., 2000), thalamus (Natsume et al., 2003) and hippocampus (Free et al., 1995). The signal intensity of the structural MR image is determined by 4 basic parameters: proton density, T1 and T2 relaxation time and flow. Proton density refers to the concentration of protons in the tissue in the form of water and macromolecules. The imaging community also refer to proton density-weighted sequences as "mixed T1/T2 weighted", the "balanced image" or the "1st echo image." The T1 and T2 relaxation times determine the way that the protons revert back to their resting states after the initial radiofrequency pulse. The most prevalent effect of flow is a loss of signal from rapidly flowing arterial blood. The most common sequences used are the T1 and T2-weighted spin echo sequences; with MR images of the brain, these are the primary determinants of signal intensity and contrast.

Computed tomography (CT) can also be used to provide good inplane resolution but in coronal CT sections, it is difficult to differentiate the hippocampi from adjacent structures such as the amygdala. Its main advantages over MRI are better visualisation of calcified tissue, lower cost and absence of distortions due to magnetic inhomogeneities. MRI has 4 main advantages over CT for assessing internal brain structures such as the hippocampus:

(1) There are none of the risks that accompany ionising radiation, thus allowing repeated and follow-up studies.

(2) MR images can be based on different types of contrast for example, T1 relaxation time, T2 relaxation time and proton density, unlike CT which has one type of tissue contrast, specific gravity. The poor soft tissue contrast of CT limits early detection of conditions such as encephalitis or ischaemic stroke while with MRI, sequences can be chosen which give maximum contrast to delineate the tissue of the structure to be analysed.

Table 4.1. MRI Sequences and Main Characteristics

Sequence	Repetition Time (msecs)	Echo Time (msecs)	CSF Contrast	Tissue Contrast
T1-weighted	<1000	<30	Dark	WM brighter than GM
T2-weighted	>1500	>60	Bright	GM brighter than WM
Proton Density	<1000	<30	Grey	GM brighter than WM

WM = White Matter; GM = Grey Matter.

(3) MRI can improve visualisation of stuctures and allow measurement reproducibility as the orientation of MR images is under operator control and can be accurately defined based on internal brain structures.

(4) MRI is superior for imaging the medial temporal lobe because CT will often produce beam hardening artefacts in the middle cranial fossa obscuring the temporal lobes. Conventional CT systems are equipped with polyenergetic X-ray sources that can prevent accurate density measurements because of beam hardening. As the beam passes through an object, it becomes harder i.e. its mean energy increases, because

lower energy photons are absorbed more rapidly than the higher energy photons. There are two types of artefact which can result from this effect: 'cupping' artefacts and the appearance of dark bands or streaks between dense objects in the image. MRI can be prone to susceptibility artefacts that show as white areas in the image and are caused when there is a disruption in the magnetic field at an air-bone interface for example, the sinus. However, these artefacts are less problematic than artefacts experienced with CT.

Despite such advantages, there are problems associated with MR imaging of acute neurological emergencies. Relatively long examination times, which can result in movement artefacts in ill and/or confused patients, and low sensitivity to acute haemorrhage, are the two main problems in an emergency clinical setting; however, these problems are not associated with follow-up studies. Conversely, CT scans are more readily available than conventional MRI and a CT scan can be more rapidly acquired for ill patients, in an environment in which it is easier to monitor the patient. Advances in MR technology have reduced examination times by employing gradient echo imaging and ultra-fast techniques such as Echo Planar Imaging (EPI) and Single Shot Fast Spin Echo (SSFSE). Other improvements include molecular diffusion and blood perfusion imaging techniques.

Therefore, because MRI has excellent spatial resolution, detailed visualisation of internal brain structures and is a non-invasive technique with no ionising radiation risks, it is superior to CT for follow-up studies of structures such as the hippocampus.

MRI and Volumetric analyses

Technological advancements in imaging, specifically MRI, have improved visualisation of internal anatomical structures *in vivo* and provided excellent resolution allowing for improved volumetry of neuroanatomy. There have been many studies carried out using MRI and volumetric analysis of brain structures such as the amygdala (Sachdev et al., 2000), fornix and mamillary bodies (Bilir et al., 1998), thalamus (Natsume et al., 2003) and hippocampus (Bernasconi et al., 2003; Baxendale, Thompson & Kitchen, 2000). Due to measurements being indirect, accuracy and validity are key issues and different measurement techniques have differing degrees of accuracy (i.e., subjected to random errors of volume measurement) or bias (i.e., a regular systematic over- or underestimation of the volume being measured) (Arndt et al., 1994). Of the different methods available for volumetric analysis of brain structures, manual-tracing techniques are one of the most commonly used.

Manual Tracing

Manual tracing techniques have been used by many groups to study a wide range of topics involving the hippocampus; the vulnerability of the hippocampus to hypoxic shock during cardiac arrest (Grubb et al., 2000), epilepsy (Bernasconi et al., 2003), Alzheimer's disease (Laakso et al., 1998; Petersen et al., 2000), schizophrenia (Sachdev et al., 2000), posttraumatic stress disorder (Villarreal et al., 2002), postoperative memory decline in epilepsy (Baxendale, Thompson & Kitchen, 2000), verbal memory performance in nondemented elderly (Hackert et al., 2002) and traumatic brain injury (Bigler et al., 1997). The quality of measurements depends on

the ability of the investigator to identify the anatomical boundaries of the hippocampus (discussed in greater detail later); the detectability of boundaries depends on resolution and grey matter-white matter contrast. Despite criticism for being labour-intensive and time consuming as well having inevitable potential inter and intra-observational measurement errors (Ashton et al., 1997; Shen, 2002), manual tracing offers sensitive and specific detection of hippocampal atrophy (Jack, et al., 1989; Cendes et al., 1993) and was the method of choice in the present study due to being readily available via Analyze, accurate and easily implemented. A recent review of hippocampal volumetrics in a database of 423 records discovered that 90% of protocols used manual tracing to determine hippocampal volume (Geuze, Vermetten & Bremner, 2005).

Thresholding

Thresholding is a method which involves selecting an intensity threshold value and then selecting a pixel in the (unmagnified) region to produce a seed. This seed point connects to neighbouring voxels with an intensity equal to or above the threshold value and a trace is automatically drawn around the region. This method can be subject to 'spillage' into other areas (Lemieux, Liu & Duncan, 2000) although this can be corrected by manual tracing of boundaries. Thresholding is not widely used for hippocampal volumetry due to the hippocampus having low contrast and discontinous edges along most of its surface. However, the continuity of the corpus callosum in mid-sagittal orientation allows thresholding to be used for area measurements (present study) and cerebellar volumetry (Liu et al., 2003).

Stereology

Stereological methods employ grids of systematically spaced crosses, which are superimposed onto each image slice and rely on the Cavalieri principle of estimating volumes where the cross-sectional area is multiplied by the slice thickness or, with regards to non-contiguous slices, multiplied by the slice thickness plus the interslice gap (Sheline et al., 1996a). This method has been used to calculate volumes of structures such as the temporal lobe (Doherty et al., 2000) putamen (Husain et al., 1991) and hippocampus (Sheline et al., 1996b). Inadequate contrast, slice thickness and partial volume effects can influence stereological volume estimates. Statistical bias can arise from the sampling design which requires starting measurements from a random starting position; this problem has often been ignored leading to biased estimates (Steinmetz et al., 1989). A false assumption is that volume is calculated independently of cross size. A study in which 3 different cross sizes were used to estimate brain volume showed a significant affect on volume attained with cross size used (Dickson, 2003). Therefore, cross size should be taken into consideration when comparing volumetric results obtained from stereology.

Semi-Automated Techniques

Semiautomated techniques include implementation of anatomical feature extraction algorithms using 2D 'snake' models (Ashton et al., 1995) or 3D models referred to as 'balloons' or deformable shape models (Shenton, 2002; Shen, 2002; Haller et al., 1997). Extraction techniques which use deformable shape models incorporate the aims behind region growing allowing incorporation of *a prioi* knowledge of hippocampal boundaries, shape and location and can approach the accuracy of

manual tracing techniques at calculating hippocampal volume (Ashton et al., 1997; Hogan et al., 2000). However, because these techniques still require user interaction, measurement errors can be made leading to variability between users and between groups (Staib, Chakraborty & Duncan, 1997).

Automated Techniques

Automated techniques such as automated elastic matching (Iosifescu et al., 1997; Andreasen et al., 1996) are useful when analysing volume changes of many different brain structures in diseases where these structures are pathologically altered simultaneously. These techniques can be used to calculate the volumes of globus pallidus, putamen, thalamus and grey and white matter in diseases such as schizophrenia (Iosifescu et al., 1997). Although it has been found that volumes for larger structures such as the thalamus obtained by this method correlate with results from manual tracing, elastic matching is not accurate for smaller, irregularly-shaped structures such as the globus pallidus and hippocampus. This is due to these structures being more sensitive to imperfections in the registration and warping steps that are used (Shen, 2002) resulting in such structures being recognised and measured with less accuracy (Iosifescu et al., 1997). Other automated techniques such as intensity-based image registration techniques (Webb et al., 1999) and high dimensional brain mapping (Haller et al., 1996; Csernansky et al., 1998; Posener et al., 2003) have been more successful in quantifying hippocampal volumes. Automated approaches to volume determination remain to be fully validated and until such times, manual or semi-automatic techniques depending on the structure of interest to be analysed, may be more appropriate.

Methodological Differences Between Studies Using Manual Tracing

MR Image Acquisition

A number of different MRI sequences with different contrasts have been used to measure the hippocampus including spin echo, fast spin echo, inversion recovery and gradient echo. Wieshmann and colleagues compared the repeatability of hippocampal volumetric measurements on two sequences with identical resolution but different contrast – gradient echo and inversion recovery. It was found that the higher grey matter-white matter contrast given by inversion recovery was associated with improved repeatability of hippocampal measurements likely due to improved detectability of hippocampal boundaries (Wieshmann et al., 1998). It is important to outline the hippocampal head correctly as it is the largest part of the hippocampus. However, this can prove difficult as it is often difficult to determine the head from the amygdala. The alveus is a thin white matter structure between the two that can aid distinction. This was shown to be more evident when using inversion recovery. However, other authors have found that differences in image acquisition and contrast have no significant effect on calculated tissue volumes (Jack et al., 1995).

Most groups use T1-weighted images although some have used T2-weighted and proton density (Pucci et al., 1998). Some authors recommend the use of T2-weighted images to distinguish between the hippocampal head and the alveus that separates the head from the amygdala (Hui, Cavazos & Tien, 1997). Other differences between groups include the angle at which the image is acquired, different boundaries being used to define the hippocampus and registering the images into standard, stereotaxic space.

It is commonly accepted that for hippocampal volumetry, the acquisition of images in the plane perpendicular to the hippocampus is preferable due to reduced partial volume effects and absence of interpolated voxels allowing superior visualisation of the temporal and frontal lobes, the hippocampus, amygdala and ventricular system (Jack et al., 1998; Bartzokis et al., 1993). Most MR imaging units are not capable of 3D oblique acquisitions but this can be overcome by positioning the subject's head to lie perpendicular to the long axis of the hippocampus. However, this position is not comfortable for the subject and can be difficult to maintain for long periods of time. Thus, most images are acquired in a non-perpendicular plane and then retrospectively reformatted for image analysis. Hasboun and colleagues (1996) compared hippocampal volumetry from images acquired in the plane perpendicular to the hippocampus, images not acquired perpendicularly and those not acquired perpendicularly but reformatted in the correct orientation and found a strong correlation between the 3 methods (Hasboun et al., 1996). Although the plane perpendicular to the hippocampus is preferable for image acquisition, the hippocampus is ideally measured in the coronal plane as the disparity of frontal and temporal lobes is most obvious. Therefore, both hippocampi can be viewed simultaneously and comparisons can be made with previous studies.

Slice Thickness

Partial volume effects, when a voxel represents more than one tissue type, can occur due to the complex nature of neuroanatomical interfaces of tissue types in the brain and can be minimised by decreasing the slice thickness. Laakso and colleagues (1997) performed hippocampal volumetry using slice thickness of 1, 3 and 5mm and

found no siginificant differences in volume and the image quality and clarity of hippocampal boundaries were not affected either. However, 'thick' slices are still not recommended as there is an increased possibility of occasional bias in total volume compared to 'thin' slices. Although some groups have used thick slices such as 5mm (Bronen & Cheung, 1991; Blatter et al., 1995), it is more usual for a slice thickness such as 1mm (Bernasconi et al., 2003; Pruessner et al., 2000) and 1.5mm (Hasboun et al., 1996; Cook et al., 1992) to be used.

Imaging Software

The method of processing the images, specifically the method of pixel counting, also contributes to the varying results found between groups. The software employed may count the pixels inside the trace, include the row of pixels under the trace in the region of interest or include the row of pixels outside of the trace in the region (Jack et al., 1995).

Image Magnification

Image magnification is an issue worth considering with respect to ROI measurements. There is a difference between image data (the actual data acquired by the scanner) and a video image (the image that is displayed on the workstation screen). Pixel counting within a defined region is performed on the image data whereas the region is manually traced on the video image. This image has to be magnified to allow the observer to accurately trace the hippocampus and this magnification can provide inconsistencies in pixel counting. Image data is typically

stored in a 256 x 256 pixel array and the diameter of the hippocampus may be about 15-20 pixels. If directly reproduced at a display screen pitch of 100 pixels per inch, the video image of the hippocampus would be 0.15-0.20 inches hence the need for magnification (Jack et al., 1995). If for example, the image data is magnified 4 times, then 16 pixels in the video image are mapped back to every one pixel in the image data. Different software packages have different ways of counting the pixels on the video image. Therefore, calculated volumes will differ accordingly. Magnifications of x3 (Free et al., 1995), x3.4 (Hasboun et al., 1996) and x4 (Lemieux, Liu & Duncan, 2000) have been reported.

Hippocampal Anatomical Boundaries

The hippocampus is anatomically closely associated with other stuctures and this has resulted in inconsistencies in the literature as to what structures to include when measuring hippocampal volume. Some groups have measured the hippocampal formation including the hippocampus, alveus and fimbria (Baxendale, Thompson & Kitchen, 2000). Others have measured the dentate gyrus, hippocampus proper and subicular complex (Laakso et al., 1998), while other groups have used measures that include the hippocampus proper, dentate gyrus, subiculum, fimbria and alveus (Hsu et al., 2002; Villarreal et al., 2002).

Different groups have used different boundaries to determine the anatomy of the hippocampus itself. There have been different criteria used to establish the anterior, posterior and inplane boundaries. Some have measured the tail and body but excluded the head (Spencer, McCarthy & Spencer, 1993), others have measured the

head and body but excluded the tail while still others have measured the hippocampus along its entire anterioposterior length (Cook et al., 1992; Bilir et al.,1998; Hackert et al., 2002). There are also differences between groups in where the measurement of the hippocampus starts and finishes. Most have opted to start at the slice where the alveus clearly delineates the hippocampus head from the amygdala (Lemieux, Liu & Duncan, 2000; Hasboun et al., 1996; Di Stephano et al., 2000) and to end on the slice where the crux of the fornix is in full profile (Hackert et al., 2002; Laakso et al., 1998; Lemieux, Liu & Duncan, 2000; Hasboun et al., 1996). Most groups measure the hippocampus in an anterior to posterior direction though some choose posterior to anterior (Hackert et al., 2002; Lemieux, Liu & Duncan, 2000).

Volume Estimation

In the past, estimates of anatomical structures were carried out using basic stereological methods that involved the area of the structure of interest being measured with a planimeter. Volumes were calculated by multiplying the area by the slice thickness and summing over all slices. This method is extremely time consuming and has since been replaced by computerised methods using digitised images and pixel counting (Arndt et al., 1994). Pixel counting is the modern version of planimetry with the same fundamental principles; a pixel represents an area located on the image slice and each slice represents a thickness. Thus, the pixel area can be transformed to a volume i.e. a voxel. In turn, the volume of the entire structure can be calculated by multiplying the number of pixels in each slice by the voxel volume and summing the results for all slices.

With regard to previously mentioned issues referring to boundary delineation, manual tracing was selected for tracing hippocampal boundaries and thresholding was chosen for corpus callosal boundaries. Volumetric measurements were calculated using pixel counting.

Neuroanatomical Volumetric Methods

MR Image Acquisition

All subjects were scanned on a 1.5 Tesla MRI scanner (Seimens, Erlangen, Germany) at the Institute of Neurological Sciences at the Southern General Hospital in Glasgow. T1-weighted images were acquired using a 3D gradient echo sequence, MP_RAGE: flip angle of 12°, a field view of 250mm and a matrix size of 256 x 256 resulting in 256 slices with a voxel size of 1.4 x 1 x 1mm^3.

Pre-processing

Pre-processing involved reformatting the MR images to the correct orientation and voxel size using the imaging processing software ANALYZE (Biodynamics Research Unit, Mayo Foundation, Rochester, MN, USA) on a SPARC 10 workstation (Sun Microsystems, Mountain View, CA, USA):

1. Conversion of images: The MR images were corrected for orientation by applying 2 rigid body translations in y and z directions to ensure that images were in the neurological orientation. The voxels were transformed to isotropic voxels using

windowed sinc interpolation (Hajnal et al., 1995) allowing fractional pixel shifts in MR data changing the voxel dimensions from 1.4 x 1 x $1mm^3$ to 1 x 1 x $1mm^3$. This interpolation was necessary to allow the subsequent reslicing of images in different planes for hippocampal volumetry.

2. Realignment of images: The 3D data set for hippocampal volumetry was realigned using the 'fusion' module of Analyze. Images were rotated to align the central line of the brain with a vertical crosshair in both transverse and coronal orientations and sagittally, the hippocampi were aligned parallel to a horizontal crosshair i.e. in a plane perpendicular to the long axis of the hippocampus.

3. Numbering: The 94 scans were numbered and to avoid order bias, the order in which the images were measured was randomly chosen using a random number generator obtained from Random.org (Haahr, 1999; www.random.org). This generates true random numbers using a source of entropy such as atmospheric noise from a radio, and is preferable to algorithms used by computer programs that can only generate pseudo-random numbers.

Hippocampal Volume Measurements

Measurements were performed using the 'Region of Interest' module in Analyze where tracing proceeded anterior to posterior. The contrast was altered for each individual image to aid visualisation of the hippocampi and surrounding structures. The following protocol was followed for each image:

1. At a low magnification, starting and finishing slices were decided. Measurement was from the hippocampal head progressing posteriorly to the hippocampal tail. The starting slice was chosen where the alveus could be most clearly seen dividing the hippocampus from the amygdala (figure 4.1). The chosen slice also showed the optic tracts coming into view and forming part of the floor of the third ventricle. These were important additional markers as the alveus was not always clearly visible.

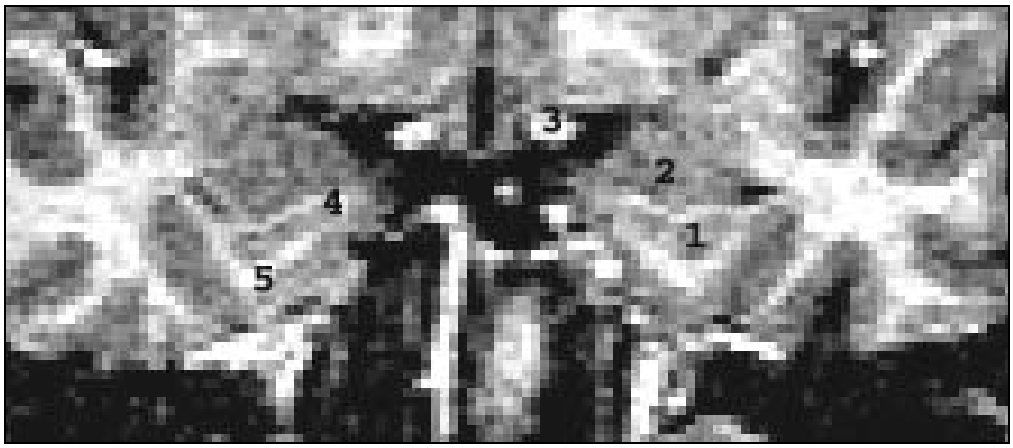


Figure 4.1. Hippocampal start slice: 1: hippocampus, 2: amygdala, 3: optic tract, 4: alveus, 5: subiculum

The hippocampal head appears as a transversely orientated oval or crescent-shaped structure and the body becomes apparent approximately at the level of the red nucleus. The level of the superior colliculus/pineal gland where the brainstem becomes separated from the midbrain is approximately where the hippocampal tail begins. The last slice to be measured was determined by visualisation of the quadrigeminal plate surrounded by CSF (figure 4.2). This slice also identifies the crux of the fornix in full profile (Hackert et al., 2002; Laakso et al., 1998; Lemieux, Liu & Duncan, 2000; Hasboun et al., 1996).

2. Images were magnified by x3 for initial measurement and then magnified by x6 to allow for detailed corrections using individual pixels. No interpolation was used when magnifying images to ensure accurate tissue representation hence the resulting pixellated images; interpolation at such magnifications would be highly inaccurate. The use of a mouse-driven cursor was sufficient for drawing regions because of the size of magnifications used. After a region was drawn for each hippocampal slice, the region was copied over to the next slice which was then adapted for that particular slice. The average number of slices measured for each hippocampus was 33.

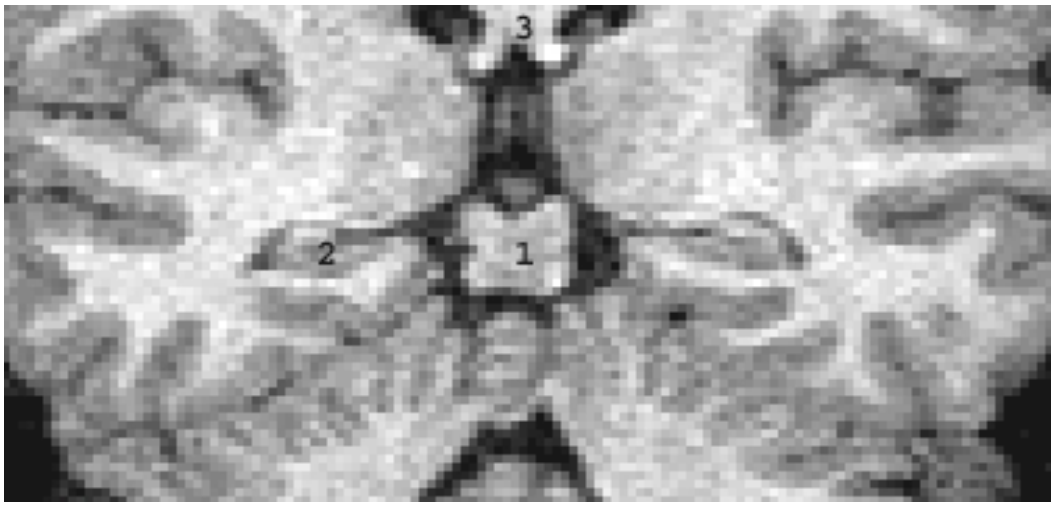


Figure 4.2. Hippocampal end slice: 1: quadrigeminal plate, 2: hippocampal tail, 3: crux of fornix

3. Hippocampal volume was calculated by the pixel counting methodology in Analyze i.e. by multiplying the number of thresholded hippocampal pixels by the voxel dimensions (1 x 1 x 1mm^3).

Corpus Callosal Area Measurements

The corpus callosum is the largest interhemispheric commissure in the brain and its area has been measured in various studies including schizophrenia (Rossell et al., 2001; Narr et al., 2002), Alzheimer's disease (Hampel et al, 2002; Teipel et al., 2002) and head injury (Levin et al., 1990a; Johnson et al., 1996).

The order of images used for corpus callosal area measurements was separately randomised using the random number generator at Random.org (Haahr, 1999). The area of the corpus callosum was measured on the midsagittal slice which was defined as the slice which showed no brain above the corpus callosum, visualisation of the pituitary stalk and showed CSF surrounding the corpus callosum (figure 4.3). If the pituitary stalk was not clear, the slice that showed the posterior commissure and pineal body surrounded by CSF was used as an alternative.

In Analyze, the Region of Interest module was used for area measurement. Rather than manually trace the area, Thresholding was employed due to the well-defined border of the corpus callosum in the mid-sagittal slice. This created a boundary of all pixels related to a specified seed point located in the corpus callosum that were within a specified intensity threshold. The threshold was adjusted to suit and the region-of-interest boundary was updated accordingly. This was performed on an unmagnified image. Minor manual adjustments were necessary and carried out on a x3 magnified image. Area measurement was calculated using pixel counting to give corpus callosum area in mm^2.

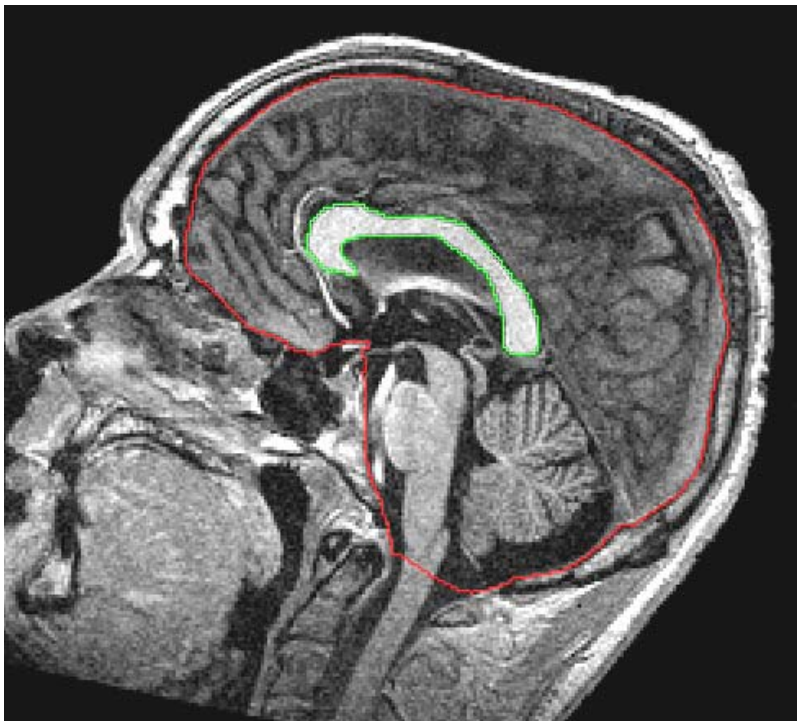


Figure 4.3. Outline of corpus callosum area (green) and intracranial area (red)

Intracranial Area

It is established that the volume of neuroanatomical structures varies among individuals as a function of head size (Gado et al., 1982; Turkheimer et al., 1984). Methods to control for such variability include the use of intracranial volume (Bigler et al., 1997; Tate & Bigler, 2000) and intracranial area (Juottonen et al., 1999; Kemppainen et al., 2003) either as a ratio measure or as an analysis of covariance approach.

Thus, to control for interindividual and intergender variability in head size, a correction factor in the form of intracranial area was applied when statistically analysing hippocampal and corpus callosal measurements. The midsagittal slices used previously for the corpus callosal area measurements were also used to define

intracranial area. The Analyze region-of-interest module was again employed for area measurement. Manual tracing began along the inner table of the cranial vault, along the superior surface of the frontal fossa floor, across the pituitary fossa to the dorsum sella and then down the posterior surface of the clivus where the area was completed by tracing along to link the anterior and posterior rims of the foramen magnum (figure 4.3) (MacLullich et al., 2002).

CHAPTER 5: NEUROANATOMICAL MEASUREMENTS

For neuroanatomical analyses, hippocampal volume, corpus callosal area and intracranial area were measured for all 47 patients at 1 and 6 months post injury by a single rater (DSR). However, for analyses of volumetric data with neuropsychological data, 1 or more patients had to be excluded due to incomplete test data. All statistical analyses were carried out using version 11 of SPSS for Windows software (SPSS, Chicago, IL, US).

Hippocampal and Corpus Callosal Volumetry

For assessment of test-retest reliability, variation in volume measurement was determined by the measurement of both left and right hippocampi of 10 random patients approximately 1 month after initial measurement of hippocampi. Intrarater reliability was assessed using intraclass correlation where reliability was found to be high for both left and right hippocampal volumetric measurement (table 5.1).

Test-retest reliability was assessed by the measurement of corpus callosa of 10 random patients approximately 1 month after initial measurement. To assess interobserver variability, a further 10 random patients were measured by a second observer, consultant neuroradiologist Professor Donald Hadley (Institute of Neurological Sciences, Southern General Hospital, Glasgow). Both intraclass and interclass reliability was found to be high with $r = 0.99$ and $r = 0.98$ respectively. The intrarater reliability of intracranial area (ICA) measurement was found to be high at $r = 0.99$ (table 5.1).

Table 5.1. Region of Interest Intraclass Reliability Coefficients

ROI	r
Left Hippocampus	0.97
Right Hippocampus	0.96
Both Hippocampi	0.95
Corpus Callosum	0.99, 0.98*
Intracranial Area	0.99

* Interclass Reliability

In order to exclude the effect of interindividual variability in head size, hippocampal volumes were normalised using intracranial area (ICA) measurements. Hippocampal volumes were divided by intracranial area to produce normalised volumes which were used in correlational analyses. Analysis showed that left hippocampal volume at 1 month had a negative association with ICA ($r = -0.32$, $p = 0.03$) illustrating the need to control for head size in the patient population.

Table 5.2. Hippocampal Volumes for 47 Head-Injured Patients

	Min	Max	Mean	SD
LHC 1	*2014.45*	*2876.85*	*2402.43*	*225.81*
LHC 1	0.11	0.2	0.15	0.02
RHC 1	*2046.11*	*2980.23*	*2460.62*	*232.33*
RHC 1	0.12	0.2	0.16	0.02
LHC 2	*2119.69*	*2890.82*	*2408.73*	*223.1*
LHC 2	0.13	0.19	0.15	0.02
RHC 2	*2125.27*	*2861.02*	*2462.69*	*212.34*
RHC 2	0.13	0.19	0.16	0.02
CC 1	*465.3*	*883.1*	*685.18*	*96.98*
CC 1	0.03	0.06	0.04	0.01
CC 2	*492.1*	*886.9*	*691.23*	*94.65*
CC 2	0.03	0.06	0.04	0.01
ICA 1	13631.8	17934.8	15708.11	990.34
ICA 2	13686.2	17987.2	15705.58	1017.19

Hippocampal volume is given in mm^3; Corpus Callosum/Intracranial Area is given in mm^2 LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum, ICA = Intracranial Area; 1 = 1 month; 2 = 6 months; Non-normalised (absolute) volume/area shown in italics

Age Effects

Age at time of injury was found to have a significant negative association with corpus callosal area at 6 months ($r = -0.38$, $p = 0.008$) and displayed the same trend at 1 month ($r = -0.28$, $p = 0.06$). No significant associations with age were found for left and right hippocampal volumes at 1 month ($r = -0.12$, $p = 0.42$ and $r = -0.13$, $p = 0.38$ respectively), 6 months ($r = -0.08$, $p = 0.59$ and $r = -0.12$, $p = 0.43$ respectively) or total volumes at 1 and 6 months ($r = -0.13$, $p = 0.39$ and $r = -0.1$, $p = 0.5$ respectively). Therefore, due to apparent age effects in the corpus callosum, in subsequent correlational analyses of neuroanatomical data, age at injury was partialled out.

To determine whether hippocampal asymmetry changed with aging over the period of 5 months, left hippocampal volume was subtracted from the right and correlated with age. The correlation was close to zero ($r = 0.09$, $p = 0.55$) thus there was no evidence of any aging effects in hippocampal asymmetry.

Gender Effects

The effect of gender on the corpus callosum is a contentious issue (Johnson et al., 1996; Sullivan et al., 2001; Constant & Ruther, 1996) as is the case with the hippocampus (Bigler et al., 1997; Raz et al., 1998; Preussner et al., 2000). The current patient group contained too few females to make meaningful comparisons on the basis of gender.

Hippocampal Asymmetry

The means show that for normalised volumes, the right hippocampus was larger than the left which was significant on ANOVA, $F(1, 46) = 21.61$, $p < 0.001$. However, there was no significant effect on hippocampal volume of time after injury, $F(1, 46) = 0.03$, $p = 0.86$. Asymmetry of hippocampal volumes has been reported elsewhere (Bernasconi et al., 2003; Tomaiuolo et al., 2004; Sullivan et al., 1995) although other authors have found no asymmetry (Bonilha et al., 2004; Baxendale, Thompson & Kitchen, 2000; Woermann et al., 1998). Handedness has been shown to affect right-to-left hippocampal volume ratios (Szabo et al., 2001). Therefore, the patient group was divided into left or right-handed subgroups and ANOVAs were carried out to determine whether handedness was a factor. Of the 47 patients, 6 were left-handed, 40 right-handed and 1 ambidextrous who was eliminated from analysis. Results showed that the right hippocampus was significantly larger than the left for both left-handers, $F(1, 5) = 22.4$, $p = 0.005$, and right-handers, $F(1, 39) = 13.85$, $p = 0.001$) demonstrating that handedness in this instance was not a factor.

Table 5.3. Neuroanatomical Correlations for 47 Patients at 1 and 6 Months

	LHC	RHC	LHC	RHC	CC	CC
	1 Month		6 Months		1 Month	6 Months
LHC 1	/	/	/	/	/	/
RHC 1	0.96**	/	/	/	/	/
LHC 2	0.86**	0.79**	/	/	/	/
RHC 2	0.87**	0.85**	0.95**	/	/	/
CC 1	0.32*	0.23	0.38*	0.36*	/	/
CC 2	0.25	0.19	0.27	0.29*	0.9**	/
ICA 1	-0.72**	-0.68**	-0.66**	-0.7**	-0.52**	-0.5**
ICA 2	-0.71**	-0.68**	-0.65**	-0.69**	-0.53**	-0.5**

LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum, ICA = Intracranial Area; 1 = 1 month; 2 = 6 months; *p < 0.05, **p < 0.01

The results of Pearson's partial correlations controlling for age calculated between each of the neuroanatomical measures are shown in a correlation matrix (table 5.3). Hippocampal volumes showed high positive correlation between left and right and at 1 and 6 months. Correlations between left hippocampi at 1 month and right hippocampi at both 1 and 6 months were similar. The same was true for the associations between right hippocampi at 1 month and left and right at 6 months. Therefore, this shows that hippocampal measurements were strongly inter-related.

Relationships Between Neuroanatomical Structures

Intracranial area at 1 and 6 months had strong negative associations with each of the neuroanatomical volumes at each of the assessments which was expected due to using ICA as a normalisation factor. Corpus callosal area showed modest but significant positive associations with each of the hippocampal volumes except CC1 with RHC1 and CC2 with LHC1 and RHC1. Corpus callosa at 1 month showed a stronger relationship with hippocampal volume at both assessments although hippocampi at 6 months had stronger associations with corpus callosa at both assessments (table 5.3).

Table 5.4. Corpus Callosal Correlations with Total Hippocampal Volume (HV)

	Total HV: 1 Month	Total HV: 6 Months
CC: 1 Month	r = 0.28, p = 0.06	r = 0.38, p = 0.01
CC: 6 Months	r = 0.22, p = 0.14	r = 0.28, p = 0.06

CC = Corpus Callosum

With regards total hippocampal volume, corpus callosal area had significant relationships with normalised total volume at both 1 and 6 months with callosal area

at 1 month having a stronger association (table 5.4). Effect sizes displaying the extent of change in terms of standard deviation, were found to be small for comparison of the structures at each assessment and were small with regard to hippocampal asymmetry although notably larger than for time of assessment. Intracranial area had an effect size of 0 indicating that intracranial area at both 1 and 6 months were extremely similar (table 5.5).

Comparison of Neuroanatomical Structures at 1 and 6 Months

Paired sample t tests showed no significant difference between hippocampal volume, corpus callosal area or intracranial area between 1 and 6 months (table 5.5).

Table 5.5. Neuroanatomical Effect Sizes

		Effect Size	t	p
LHC 1	LHC 2	0.03	-.27	.79
RHC 1	RHC 2	0.01	-.09	-93
CC 1	CC 2	0.06	-1.76	.09
Total Volume 1	Total Volume 2	0.02	-.18	.86
ICA 1	ICA 2	0.00	.15	-88

LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum, ICA = Intracranial Area; 1 = 1 month; 2 = 6 months

Changes in hippocampal absolute (non-normalised) volumes for individual patients can be seen in figure 5.1. Individually, the total hippocampal volumes of patients included a combination of increases and decreases. Mean absolute volume increased over the 5-month period by 0.2 % from 4863.05 mm^3 to 4871.42 mm^3. However, a paired sample t test showed this increase to be non-significant, t (46) = -0.18, p = 0.86. The hippocampal volumes are within range of those reported elsewhere in the literature. For example, Bigler and colleagues reported 5550 mm^3 for control

subjects and 4720 mm^3 for head injured subjects imaged more than 90 days post-injury (Bigler et al., 1996). Means of 4923 mm^3 for control subjects and 4415 mm^3 for head injured subjects imaged at least 2 months post-injury have been reported (Tate & Biger, 2000).

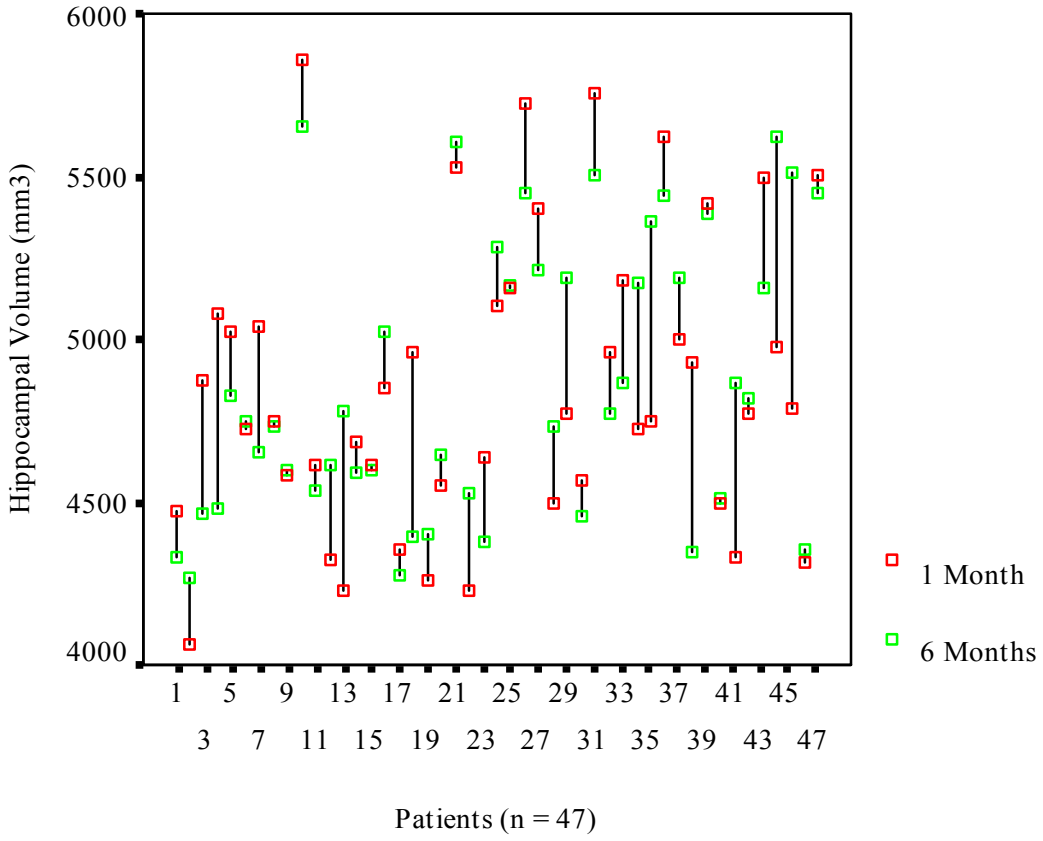


Figure 5.1. Changes in absolute total hippocampal volume

Changes in absolute corpus callosal areas for individual patients are illustrated in figure 5.2. Mean corpus callosal areas at 1 and 6 months were 685.18 mm^2 and 691.23 mm^2 respectively. These are in agreement with callosal area reported elsewhere; for example, 689.18 mm^2 (Dorion et al., 2000) and 627 mm^2 (Mitchell et al., 2003). Although the mean corpus callosal area increased from 685.18 mm^2 to

691.23 mm^2, an increase of 0.88 %, a paired sample t test illustrated that this was not significant; t (46) = -1.76, p = 0.09.

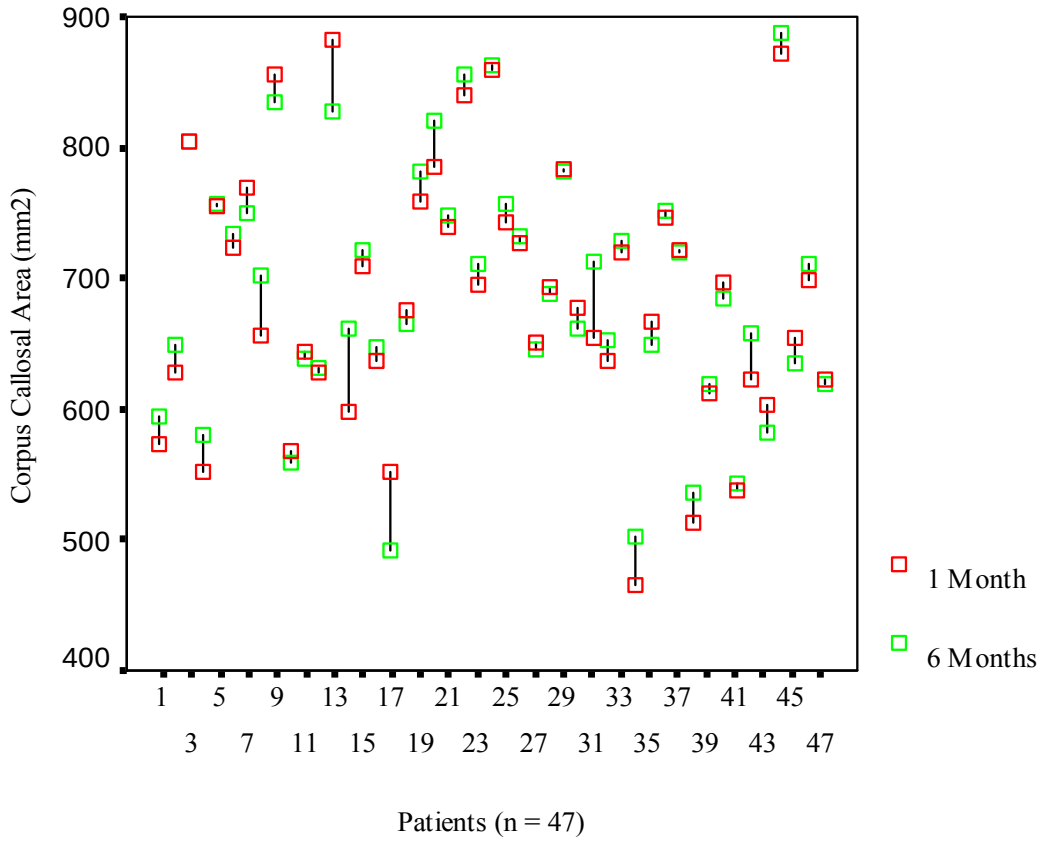


Figure 5.2. Changes in absolute corpus callosal area

Neuroanatomy and Glasgow Coma Score

Spearman correlations revealed significant positive associations between the left and right hippocampi at 1 month with the 24-hour worst GCS score. The left hippocampus at 1 month also showed a positive correlation with the GCS at A&E score. The left hippocampus showed a marginally stronger association with each GCS classification than the right hippocampus. The right hippocampus displayed the

same trends as the left hippocampus with regards GCS classification although fewer reached statistical significance (table 5.6).

Table 5.6. Neuroanatomical Correlations with GCS

	LHC	RHC	LHC	RHC	Corpus Callosum	
	1 Month		6 Months		1 Month	6 Month
GCS at A&E	$r_s = .34$*	$r_s = .28$	$r_s = .28$	$r_s = .27$	$r_s = -.03$	$r_s = -.1$
(n = 47)	p = .02	p = .06	p = .06	p = .08	p = .84	p = .94
GCS 24 Hour	$r_s = .36$*	$r_s = .32$*	$r_s = .32$*	$r_s = .28$	$r_s = -.06$	$r_s = -.06$
(n = 40)	p = .03	p = .04	p = .04	p = .09	p = .83	p = .72
GCS & CT	$r_s = -.22$	$r_s = -.17$	$r_s = -.17$	$r_s = -.2$	$r_s = .06$	$r_s = .03$
(n = 47)	p = .15	p = .26	p = .26	p = .18	p = .67	p = .83

LHC = Left Hippocampus, RHC = Right Hippocampus; *p < 0.05 (1-tailed)

Correlations of hippocampal volume and GCS & CT severity ratings were explored, as this GCS classification is more likely to reflect focal injury that may have affected hippocampal volume. No correlations reached statistical significance suggesting that diffuse injury was more likely. Corpus callosal correlation with GCS showed no significant association with any of the 3 indices of injury severity.

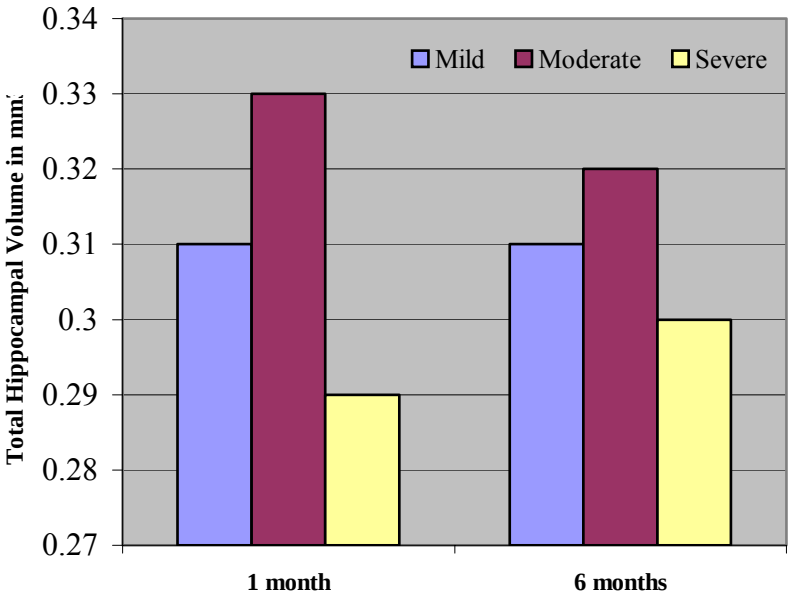


Figure 5.3. Injury severity defined by GCS at A&E and total hippocampal volume

Patients were divided into 3 groups using GCS at A&E severity ratings to give a group of mild (n = 36), moderate (n = 4) and severe GCS (n = 7). Although total hippocampal volumes at 1 and 6 months were very similar, figure 5.3 suggests that marginally larger hippocampal volumes at both 1 and 6 months post injury are associated with mild to moderate GCS severity rating. However, further analysis using one-way ANOVAs showed the associations with GCS at A&E and total hippocampal volume at 1 and 6 months to be non-significant with $F(46) = 1.13$, $p = 0.33$ and $F(46) = 0.26$, $p = 0.77$, respectively.

Neuroanatomy and Neuropsychological Test Scores

Pearson's partial and Spearman's correlations were used to determine associations between neuroanatomical structures and neuropsychological test scores and outcomes. Error scores for the National Adult Reading Test (NART) were not found to correlate significantly with neuroanatomy but significant associations were found with a proportion of test scores at 1, 3 and 6 months (table 3.15). Significant correlations were also found between age and a selection of cognitive test scores (BVRT, SDMT written and the Rey Figure). Therefore, subsequent Pearson's correlational analysis with test scores partialled NART error scores as well as age at injury to avoid potential confounding effects.

Relationships between regions-of-interest and cognitive test data were investigated using Pearson correlations (table 5.7). Correlations were performed between neuroanatomy at 1 month and cognitive data at 1 month and between neuroanatomy at 6 months and cognitive data at 6 months. Such analyses allowed for relationships

to be investigated at each of the specific assessments. At 1-month assessment, there were no significant associations between neuroanatomy and story recall, digit span, BVRT or the Grooved Pegboard using the dominant hand. Significant negative correlations were found for the Grooved Pegboard using the non-dominant hand with the left hippocampus giving a slightly stronger association than the right hippocampus. Although not significant, this trend with the hippocampi was also observed for the Grooved Pegboard using the dominant hand.

Table 5.7. Pearson Correlations with Tests Administered at 1 Month and 6 Months

	N	LHC	RHC	LHC	RHC	CC	CC
		1 Month		6 Months		1 Month	6 Months
1 Month Scores							
Immediate Story Recall	46	0.02	0.10	/	/	-0.19	/
Delayed Story Recall	46	0.12	0.20	/	/	-0.06	/
Digit Span	46	-0.10	-0.70	/	/	0.11	/
BVRT	46	-0.11	-0.07	/	/	-0.15	/
Pegboard (D)	44	-0.19	-0.15	/	/	-0.03	/
Pegboard (ND)	44	-0.39*	-0.3*	/	/	-0.12	/
6 Month Scores							
Immediate Story Recall	44	/	/	0.24	0.3*	/	0.26
Delayed Story Recall	46	/	/	0.27	0.33*	/	0.29
Digit Span	46	/	/	-0.22	-0.21	/	0.13
BVRT	46	/	/	-0.6	-0.09	/	-0.19
Pegboard (D)	44	/	/	-0.24	-0.20	/	-0.14
Pegboard (ND)	44	/	/	-0.43**	-0.35*	/	-0.24

LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum
*p < 0.05 level, **p < 0.01 level; ND = Non-Dominant Hand, D = Dominant Hand

Pearson correlations with 6-month neuroanatomy and 6-month test data showed significant positive associations with story recall scores and the right hippocampus. The association of story recall at 1 and 6 months post injury with corresponding hippocampal measurements at 1 and 6 months post injury displayed a trend whereby

strength of association was marginally stronger with the right hippocampus. Also, associations of story recall with hippocampal measurements were stronger at 6 months. Analysis of the 6-month Grooved Pegboard score with 6-month neuroanatomy revealed significant correlations of the non-dominant hand score with the left and right hippocampi. There was a weak trend observed for both dominant hand and non-dominant hand scores whereby associations with the right hippocampus were marginally stronger than those with the left hippocampus. This weak trend was also observed for correlations between the Grooved Pegboard scores at 1 month and hippocampal measurements at 1 month. As with 1-month assessment scores, correlations between 6-month neuroanatomy and 6-month digit span and BVRT were not significant. No significant correlations were found with corpus callosal area and cognitive test data at either 1 or 6-month assessments.

Table 5.8. Pearson Correlations with Tests Administered at 6 Months only

	N	LHC	RHC	CC
		6 Months		6 Months
SDMT Written	46	0.22	0.22	0.15
SDMT Verbal	46	0.33*	0.35*	0.11
Rey Figure	46	0.19	0.21	0.07
COWAT	46	0.16	0.24	0.10
TMT A	46	-0.20	-0.16	-0.21
TMT B	46	-0.21	-0.18	-0.23
MSRT Movement	40	-0.16	-0.11	0.04
MSRT Choice	40	0.07	0.02	0.12
MCRT Movement	40	-0.14	-0.12	-0.01
MCRT Choice	40	-0.21	-0.19	0.22

LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum
*$p < 0.05$ level

Of the tests administered solely at 6 months post injury, correlations of 6-month neuroanatomy with the verbal component of the SDMT were shown to be significant; there was positive association of verbal score with the left and right

hippocampal volumes. A general trend was evident whereby the right hippocampal volume tended to provide marginally weaker associations with cognitive test scores compared to the left hippocampal volume. This was true for all tests at 6 months except the SDMT, the Rey Figure and the COWAT; here, associations with the right hippocampus were marginally stronger. With regards corpus callosal area, there were no significant associations with 6-month callosal area and data from the cognitive tests administered solely at 6 months.

Neuroanatomy and Neuropsychological Outcome Measures

Neurobehavioural Functioning Inventory

Patient scores for the NFI subscales were correlated with ROI measurements resulting in only one significant association; the 6-month somatic score was significantly associated with corpus callosal area at 6 months suggesting that smaller callosal area is associated with a greater number of symptoms. Generally, there was a trend towards an increase in association with NFI score from 1 month to 6 months with hippocampal volume and corpus callosal area. However, despite a single significant correlation, ultimately there was no relationship with the NFI symptom checklist and neuroanatomical measures.

Table 5.9. Spearman Correlation of NFI Scales with ROI

	LHC 1	RHC 1	LHC 2	RHC 2	CC 1	CC 2
1 Month						
Depression	0.1	0.14	/	/	0.001	/
Somatic	-0.12	-0.16	/	/	-0.14	/
Memory/Attention	-0.01	-0.01	/	/	-0.01	/
Communication	0.09	0.01	/	/	0.16	/
Aggression	0.11	0.13	/	/	-0.18	/
Motor	-0.16	-0.1	/	/	-0.11	/
6 Months						
Depression	/	/	0.11	0.15	/	-0.04
Somatic	/	/	-0.16	-0.13	/	-0.35*
Memory/Attention	/	/	0.14	0.19	/	-0.03
Communication	/	/	0.09	0.15	/	0.04
Aggression	/	/	0.11	0.18	/	-0.05
Motor	/	/	0.03	0.1	/	-0.24

LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum
1 = 1 month; 2 = 6 months; *p < 0.05 (1-tailed)

Hospital Anxiety and Depression Scale

Scores on the HADS were correlated with anatomical measurements using 1-tailed non-parametric Spearman correlations. Only one correlation reached statistical significance, 6-month corpus callosal area was negatively associated with depression at 6 months suggesting that a smaller callosal area is associated with higher levels of depression. There is a weak trend towards stronger association of neuroanatomical measures with depression at both 1 and 6 months than anxiety (table 5.10) but ultimately it can be assumed that there is no relationship between neuroanatomy and the HADS.

Table 5.10. Spearman Correlation of HADS with ROI

	N	Anxiety		Depression	
		1 Month	6 Months	1 Month	6 Months
LHC 1	41	$r_s = 0.01$	/	$r_s = 0.05$	/
RHC 1	41	$r_s = 0.02$	/	$r_s = 0.04$	/
LHC 2	41	/	$r_s = -0.07$	/	$r_s = 0.03$
RHC 2	41	/	$r_s = 0.03$	/	$r_s = 0.1$
CC 1	41	$r_s = -0.06$	/	$r_s = 0.07$	/
CC 2	41	/	$r_s = -0.19$	/	$r_s = -0.27$*

LHC = Left Hippocampus, RHC = Right Hippocampus, CC = Corpus Callosum
1 = 1 month; 2 = 6 months; *$p < 0.05$ (1-tailed)

Discussion

Summary of Results

Hippocampal asymmetry was such that the right hippocampus was significantly larger than the left hippocampus. This has been widely reported in the literature as has age effects in neuroanatomical structures. The current study found modest correlation with age and the corpus callosum at 6 months after injury and a similar trend at 1 month. With regards volumetric change between 1 and 6 months after injury, there was no change in hippocampal volume or corpus callosal area as a result of head injury. In relation to injury severity, significant correlations with the GCS and the hippocampus were modest with a trend indicating marginally stronger association with the left hippocampus and with both hippocampi at 1 and 6 months. Correlation of neuroanatomy with behavioural symptoms as measured by the NFI and the HADS resulted in little or no association. However, the few significant correlations with the corpus callosum were in the expected direction with smaller area being associated with a greater number of symptoms. In relation to

neuropsychological test scores, there were no significant correlations with the corpus callosum and only a small number with the hippocampus suggesting that reduced cognitive performance was associated with smaller hippocampal volumes.

The following discussion starts by discussing the hippocampus and corpus callosum with regard to head size, age, gender and laterality before continuing with a discussion regarding the anatomical measures in relation to injury severity, behavioural outcome, memory and cognitive performance. Finally, limitations regarding volumetric analysis are offered.

Neuroanatomical Association with Head Size and Age

Advantages of magnetic resonance imaging such as increased availability and high spatial resolution has led to widespread research on neuroanatomical structures such as hippocampus and corpus callosum *in vivo*. It has been established that variation in head size has a direct relationship on brain size and therefore brain structures (Gado et al., 1982; Turkheimer et al., 1984). Most studies have controlled for head size in corpus callosal measurements (Levin et al., 1990a; Tomaiuolo et al., 2004) and hippocampal measurements (Bigler et al., 1997; Kemppainen et al., 2003) and the current study supports this, as significant correlations with ICA were found with left hippocampal volume which validates the need to control for such interindividual variability.

With regards age, the present study found a modest association with corpus callosum at 1 and 6 months post injury suggesting that as age increases, callosal area

decreases. This is in agreement with other studies that have found modest changes (Driesen & Raz, 1995; Johnson et al., 1994; Pozzilli et al., 1994). Although not significant, hippocampal volumes showed the same inverse trend with regard to age. The age span of the patient group was 16-66 years and the absence of hippocampal tissue loss as a function of age has also been reported in a head injured group with an age range of 16-65 years (Bigler et al., 1997) and in a normal male population with an age range of 21-70 years. These results may appear to be in conflict with what is known regarding aging and neuroanatomy but the age span used must be taken into consideration and provides a possible explanation. Hippocampal volume loss has not been detected when analysing healthy controls with an age range of 20-53 years (Jack et al., 1989; Free et al., 1995) whereas atrophy has been detected in healthy controls in studies of Alzheimer's disease which typically have an older age range for example, 64-79 years (Juottonen et al., 1999). The merger of these results suggests that hippocampal volumes remain steady from young adulthood, through middle age until later in life when atrophy begins (Jack, 1997). Therefore, it is quite conceivable that the absence of age effects in the hippocampus in the current study is due to having few sujects post-64 years of age.

Neuroanatomical Association with Gender

Gender-based morphologic differences in the hippocampus and corpus callosum are still a contentious issue with no agreement on sexual dimorphism. With regards to the corpus callosum, Dubb and colleagues (2003) reported females having a larger splenium but a smaller genu than males in a sample of healthy volunteers with an age range of 18-84. It was also suggested that changes in the corpus callosum due to age

were also gender specific with female splenia expanding with age and male genu contracting with age (Dubb et al., 2003) although others have found that age is not significantly associated with corpus callosal size (Sullivan, Marsh & Pfefferbaum, 2005). Larger splenia in females than males has also been linked with better cognitive performance in neuropsychological tasks (Davatzikos & Resnick, 1998). Others have found larger corpus callosal area in females compared to males when brain size is taken into consideration (Johnson et al., 1996; Mitchell et al., 2003) while still others have found the opposite to be true (Sullivan et al., 2001) or have found no sexual dimorphism at all (Constant & Ruther, 1996). These inconsistent results are due in part to the differing methodologies used to gain callosal measurements (Bermudez & Zatorre, 2001).

Gender differences in the hippocampus have also yet to be confirmed with studies providing conflicting evidence for sexual dimorphism. Some studies have reported that after head size correction, females had larger hippocampi than males (Bigler et al., 1997; Filipek et al., 1994) while others have found no gender-based differences in hippocampal volume when correcting for head size (Bhatia et al., 1993; Raz et al., 1998; Preussner et al., 2000).

The current study had insufficient female numbers to make any sensible analysis and conclusion of gender effects on hippocampal and corpus callosum neuroanatomy. The resolution of the debate regarding sexual dimorphism in these structures would be aided with common and consistent methodology.

Hippocampal Laterality

Hippocampal volume loss and its correlation with other structures and neuropsychological function has resulted in studies in many areas of brain injury including epilepsy (Bernasconi et al, 2003), memory impairment (Grubb et al., 2000), Alzheimer's disease (Jack et al., 1992), Schizophrenia (Narr et al., 2004) and post-traumatic stress disorder (Villarreal et al., 2002). Hippocampal laterality in such studies has varied with some reporting no effects of asymmetry (Bonilha et al., 2004; Baxendale, Thompson & Kitchen, 2000; Woermann et al., 1998) and others finding larger right than left hippocampal volume (Bernasconi et al., 2003; Tomaiuolo et al., 2004; Sullivan et al., 1995). The finding that right hippocampi are significantly larger than left hippocampi provides further evidence of hippocampal asymmetry. Although the current study found no decrease in volume from 1 to 6 months post injury, bilateral hippocampal reductions have been found in head-injured populations compared to controls (Arciniegas et al., 2001; Bigler, Anderson & Blatter, 2002; Bigler et al., 1997).

Neuroanatomical Association with Injury Severity

Callosal morphology has been studied in relation to dyslexia (Rumsey et al., 1996) and schizophrenia (Narr et al., 2002; Keshavan et al., 2002) and atrophy of the corpus callosum has been found in post-traumatic stress disorder (Villarreal et al., 2004) and Alzheimer's disease (Teipel et al., 2002; Dorion et al., 2002). Damage resulting from microscopic lesions caused by head injury is most often associated with frontal and temporal lobes, the anterior commissure and the corpus callosum. White matter tracts such as the corpus callosum are susceptible to rotational forces,

shearing, tensile effects, axotomy and Wallerian degeneration (Graham, Gennarelli & McIntosh, 2002). Due to its midline location, the corpus callosum is vulnerable in head injury due to these physical effects allowing it to be a sensitive indicator of brain atrophy (Johnson et al., 1996).

The current study looked at corpus callosal area at 1 and 6 months post injury and found no significant change in size over the 5-month period suggesting that white matter atrophy due to diffuse axonal injury had not significantly occurred. This is inconsistent with previous research which demonstrates posttraumatic callosal atrophy after head injury in relation to controls (Tomaiuolo et al., 2004; Johnson et al., 1996; Levin et al., 1990a). Studies have suggested that callosal atrophy is related to injury severity (Gale et al., 1995). However, it has also been found that although a patient group with mean GCS score of 8 or less had decreased callosal area in relation to chronicity, it was not related to severity of injury (Levin et al., 1990a).

Time dependence of white matter degenerative changes is important to consider when analysing corpus callosal atrophy as pathology can take a considerable period of time to manifest. This may go some way to explaining discrepancies between studies which have found significant correlation of corpus callosal measurements with clinical status. Patients scanned at 60 days post injury were found to have limited clinical importance with callosal measurements (Henry-Feugeas et al., 2000) whereas patients who had been scanned after a longer time period of 116 days, had enhanced correlations with clinical measures (Gale et al., 1995). The current study investigated callosal correlation with 3 Glasgow Coma Scale scores: administered at A&E, worst 24 hour GCS and GCS CT, which followed the conventional GCS

banding except that GCS 13-15 was classed as moderate if there was an abnormal CT. There were no significant associations of callosal area with any of the GCS scores which is in contrast with previous findings where injury severity as measured by GCS has been attributed to callosal area deficit (Gale et al., 1995).

Correlation of GCS along with gradual degenerative change in callosal area may have been expected in the current study, especially in images at 6 months post injury. Absence of such changes may reflect the injury severity of the patient group; in this instance, the majority of the patients (77 %) had a GCS at A&E score of 13-15 indicating mild head injury. MR images were visually inspected for the presence of lesions at corpus callosa and no lesions could be visually detected. Thus, it is argued that pathological change in the form of DAI in the patient group was not substantial enough to be associated with GCS score or detected by area measurements.

Hippocampal atrophy as a result of traumatic brain injury has been reported in animal models (Pierce et al., 1998; Hicks et al., 1993; Kotapka et al., 1991). The temporal lobe is vulnerable to mechanical injury because of its position in the middle cranial fossa. In addition to physical force, further hippocampal cellular damage can be produced in the form of exocytotoxic reactions (White & Reynolds, 1996) and/or cell death as a result of hippocampal deafferentation and/or de-efferentation (Gennarelli, Thibault & Graham, 1998). A previous study by Bigler and colleagues (1997) which focused on hippocampal volumes after TBI found that patients in the late group (imaged more than 100 days after injury) had significantly reduced hippocampal volumes than those in the early group (imaged up to and including 100 days after injury) when compared to controls (Bigler at al., 1997). This is in contrast

to the current study which found no change in volume within the patient group as a function of time. However, the main difference between the two studies was that of mean GCS. Bigler et al. reported a mean initial GCS of 7.97 for the early group and 7.12 for the late group indicating a severely head-injured population; the mean GCS for the current study was 13.1 indicating a more mildly head-injured population. Hence, this variation may account for the lack of hippocampal change at the 6-month stage as hippocampal atrophy may be linked to severity of injury. However, the results of Bigler et al. do support the current finding that GCS was positively correlated with hippocampal size. Other studies using patient groups with a mean GCS of 8.4 and <8 and imaged at least 2 months post injury, reported hippocampal atrophy when compared to controls (Tomaiuolo et al., 2004; Tate & Bigler, 2000) and found severity of injury to be related to degree of atrophy (Tate & Bigler, 2000).

The Glasgow Coma Scale is particularly discriminatory of diffuse brain injury. GCS score has been shown to correlate with hippocampal volume in patients with a mean GCS of 7.12 imaged >100 days after injury (Bigler et al., 1997) and in a group with a mean GCS of 8.4 imaged at least 2 months after injury (Tate & Bigler, 2000). The current findings showed correlation of bilateral hippocampal volume at 1 month with 24 hour worst GCS, left hippocampal volume at 1 month with GCS at A&E and left hippocampal volume at 6 months with 24 hour worst GCS. The remaining correlations were close to significance and followed the same trend. Therefore, the current results suggest that the hippocampus is more susceptible to diffuse brain injury than the corpus callosum where there were no significant associations with GCS.

Cecil and colleagues (1998) demonstrated that Magnetic Resonance Spectroscopy (MRS), a non-invasive method of investigating neurochemical changes in disease and injury in the brain, may be a more sensitive method to detect diffuse axonal injury in head injured patients. Metabolic changes of N-acetyl aspartate, which may reflect axonal injury, was found in the splenia of mildly head injured patients (Cecil et al., 1998) suggesting that decreases of this particular metabolite may be an indicator of pathological conditions which cannot be detected by region of interest methodology such as the semi-automated method used in the current study.

Neuroanatomical Association with Outcome Measures

In the neuroendocrine theory of depression, the hippocampus is an important structure. The hypothalamo-pituitary-adrenal axis raises glucocorticoid levels in the event of stress and results in a downregulation of glucocorticoid receptors if this stress is chronic. The hippocampus is sensitive to endogenous glucocorticoid levels and sustained high levels may be toxic to the hippocampus inhibiting neurogenesis (Sheline et al, 1996b); once a patient is in remission, these levels return to normal. There is disagreement with regards depression and hippocampal irregularities as some have demonstrated smaller volumes in currently depressed patients (Sheline et al, 1996b; MacMaster & Kusumakar, 2004) while others have found reduced left hippocampal volume in patients in remission (Bremner et al, 2000; Sheline, 2003) and still others have reported no differences in volume between depressed patients and healthy controls (Axelson, 1993). The current study found that the right hippocampal volume at 6 months was associated with the depression component of the NFI at 1 month and also the depression component of the HADS at 1 month.

Few studies have investigated depression with regard to corpus callosum morphology. One study that looked at major depressive disorder found no significant differences in callosal morphology between patients and controls although patients with familial major depressive disorder were reported to have larger genu and splenia in relation to patients with non-familial major depressive disorder (Lacerda et al., 2005). The current study found no association with callosal area and the depression component of the NFI but significant associations with the depression category of the HADS at 6 months with callosal area at 1 and 6 months post injury.

The current study looked at depression using the HADS and as a subcomponent of a broader questionnaire (NFI). As the patients in question were head injured rather than clinically depressed, it would be unwise to compare results with studies which have used clinically depressed populations. Also, with regard to the NFI, the correlation matrix returned a total of 72 correlations. With significance at $p < 0.05$, it would be expected that circa 3 of these 72 correlations would be false positives. Thus, this must be taken into consideration when interpreting these results. However, depression after head injury has been widely reported in the head injury literature (Kreutzer, Seel & Gourley, 2001; Seel et al., 2003; Jorge et al., 2004).

Hippocampal Volume and Association with Memory

Due to the link with the hippocampus and related medial temporal lobe structures in relation to explicit memory, many clinical studies have focused on and gained quantitative evidence, to show relationships with hippocampal volume and neuropsychological indices of memory (Bigler et al., 1996; Tate & Bigler, 2000;

Petersen et al., 2000). In this case, memory was specifically investigated using immediate and delayed story recall, the Benton Visual Retention Test (BVRT) and delayed recall of the Rey Figure. At 1 month post injury, there were no correlations of hippocampal volume with any of the memory test scores. BVRT and Rey Figure score at 6 months also had no associations with hippocampal volume. However, immediate and delayed recall at 6 months correlated with both left and right hippocampal volume at 1 month and with right hippocampal volume at 6 months post injury indicating that lower recall score is associated with reduced hippocampal volume. Thus, this suggests that hippocampal damage was sufficient enough to produce impaired story recall.

There is evidence to suggest that lateralisation of function is found in the hippocampus. Evidence suggests that the left hippocampus is involved with verbal memory (Frisk & Milner, 1990) and the right hippocampus is involved in non-verbal memory such as visual and spatial memory (Smith & Milner, 1981). Lateralised function of the hippocampus has been demonstrated in Alzheimer's patients (Petersen et al., 2000) and epilepsy (Baxendale, Thompson & Kitchen, 2000). Considering this information, it would perhaps seem counterintuitive that right hippocampal volume at 6 months would positively correlate with story recall at 6 months. However, the evidence in the research literature is inconclusive and the literature concerning hippocampal damage in relation to head injury and neuropsychological testing is limited. Some studies using severely head-injured populations (GCS <8) have found correlations of verbal memory with both right and left hippocampal volumes imaged 71-210 days injury (Bigler et al., 1997) or have found no association of immediate and delayed story recall with hippocampal

volume (Tomaiuolo et al., 2004). Other research has reported significant atrophic changes in the left but not the right hippocampus in a brain-injured population imaged 90 days after injury (Bigler et al., 1996). In view of these latter investigations, the current finding that left and right hippocampal volume at 1 month and right hippocampal volume at 6 months post injury are associated with recall supports the view that verbal and non-verbal memory are not completely lateralised in the hippocampus.

An alternative explanation could be reorganisation of memory function. It has been demonstrated in free recall tests with commissurotimised patients that the medial temporal lobes communicate with one another (Dobbins et al., 1998). Therefore, it could be postulated that atrophy to either hippocampus results in compensation for detriment to the other albeit less efficiently (Pillon et al., 1999). This may explain the current findings where the relationship of recall with the right hippocampus is stronger, albeit modestly so, than recall with the left hippocampus. It may be that although not substantial enough to be detected by region of interest methodology, atrophy in the left hippocampus had occurred enough to allow the right hippocampus to assume a greater role in immediate and delayed recall. This compensational role of the hippocampus is given credence due to the increased severity of underperformance in specific types of memory after bilateral compared to unilateral temporal lobe lesion (Warrington & Duchen, 1992). Warrington & Duchen (1992) reported results of a patient who had previously undergone right temporal lobectomy for intractable epilepsy and was subsequently severely amnesic. Encephalography and angiography was unsuccessful in detecting any abnormality in the left temporal lobe which led to speculation that memory function may be lateralised to one

temporal lobe. However, at autopsy a lesion in the left hippocampus was discovered indicating that bilateral lesions were indeed the cause of the patient's severe amnesic state.

Also, after left hippocampal atrophy for example, the patient does not lose the ability of verbal memory indicating that the right hippocampus may be involved, perhaps to a greater degree than pre-injury. The fact that verbal or visuo-spatial memory is not lost following respective hippocampal pathology provides evidence to what is known about memory processes involving a number of temporal lobe structures. With regards head injury, memory deficit is likely to be caused by diffuse damage to these structures and perhaps the hippocampus in particular.

It has been suggested that it is the head of the hippocampus which is involved in verbal memory as both the left and right hippocampal head was shown to be positively associated with immediate and delayed recall in a non-demented elderly population (Hackert et al., 2002). The hippocampal head has also been shown to more atrophied than the body or tail in temporal lobe epilepsy (Bernasconi et al., 2003). It was outwith the scope of this study to measure the hippocampal head, body and tail as separate entities. However, further research in this area with regard to head injury would allow discrimination of hippocampal sub-components with regard to memory function.

The current study investigated visuo-spatial memory by using the Rey Figure and the Benton Visual Retention Test (BVRT). No associations were found with hippocampal volume and the Rey Figure score or BVRT score. One possible

explanation is that tests used to assess visuo-spatial memory such as the Rey Figure and the BVRT are more difficult for the patient than tests assessing verbal memory. As a result, non-verbal memory tests are more prone to non material-specific components (Barr et al., 1997). Results suggesting lateralisation of visuo-spatial memory in the right hippocampus are less common than studies reporting lateralisation of verbal memory in the left hippocampus and are no less conflicting.

Although still controversial, the general consensus is that the right hippocampus is associated with non-verbal memory such as visuo-spatial memory; the right hippocampus has been associated with Rey Figure recall in severe TBI patients (Tomaiuolo et al., 2004), with BVRT score in subjects with age-associated memory impairment (Soininen et al., 1994) and greater spatial recall has been demonstrated after left temporal resection (Pillon et al., 1999). However, debate still surrounds this issue. Previous studies have indicated involvement of both temporal lobes where both left and right Mesial Temporal Sclerosis (MTS) patients had poor recall of the Rey Figure compared to controls (Miller, Munoz & Finmore, 1993) while others have found no association with the right hippocampus and visuo-spatial recall using the Rey Figure (Kilpatrick et al., 1997) and no association of visual memory with either hippocampus (Bigler et al., 1997).

With regards the corpus callosum, the current study did not find significant correlations with any of the indices of memory either at 1 or 6 months post injury. Previous research has shown corpus callosum volume to be significantly correlated with immediate free recall of word lists (Tomaiuolo et al., 2004).

The current study found no associations of neuroanatomy with digit span which measures attention and short-term verbal memory. This supports previous research which has found digit span in a group of amnesic patients with hippocampal damage to be similar to that of controls (Cave & Squire, 1992) and in head-injured patients where there were no differences compared to controls (Nissley & Schmitter-Edgecombe, 2002). These results would be expected as digit span is thought to relate to frontal lobe dysfunction rather than temporal lobe damage. However, there is evidence to the contrary, a group of severely head-injured patients performed worse on the backwards digit span compared to controls (Schmitter-Edgecombe & Woo, 2004).

To assume that memory disorders are due to deterioration of a specific structure in the brain such as the hippocampus is over-simplistic (Bigler et al., 1996) as the hippocampus is only part of a complicated network regulating memory. Rather, a multifaceted relationship between memory and damage is likely to exist. Diffuse damage in closed head injury is likely to disrupt the neural circuitry in several processing systems such as information processing and storage and retrieval of memory at different levels (Tomaiuolo et al., 2004).

Neuroanatomical Association with Neuropsychological Test Scores

Reaction Time tests were used to detect changes in processing speed which may have been compromised as a result of diffuse damage. Although the current study did not find any significant correlation between corpus callosal area and reaction time, slower reaction times have been reported in patients with mild, moderate and severe

traumatic injury when compared to controls suggesting lower processing speed as a result of diffuse damage (Mathias, Beall & Bigler, 2004; Mathias et al., 2004). In the corpus callosum, the amount of damage has been related to injury severity where severe head injured patients differed in callosal measures compared to mild and moderate head injured patients (Gale et al., 1995). This offers an explanation for the current findings by suggesting that injury was not sufficiently severe to cause changes in the corpus callosum. Also, as previously stated, it may also be that damage to the corpus callosum was not of a sufficient magnitude to be detected by the applied region-of-interest methodology.

Verbal fluency was measured using the Controlled Oral Word Association Test (COWAT). Previous research has found reduced COWAT performance in moderate and severely injured patients compared to controls (Mathias et al., 2004; Schmitter-Edgecombe & Woo, 2004) and, although not significant, a trend has been demonstrated in very mild head injured patients (Voller et al., 1999) although mild head injured patients have also shown no deficit in COWAT score (Mathias, Beall & Bigler, 2004). In the current study, there were no significant associations with COWAT and neuroanatomy which was not unexpected as the COWAT test is thought to relate to frontal lobe dysfunction rather than temporal lobe damage.

Visual-motor integration and mental processing speed were assessed using the Grooved Pegboard, Symbol Digit Modalities and Trail Making Tests. Severely head-injured patients have been found to give poorer performances on Trail Making test A, the Digit Symbol subtest of the WAIS-R and the Symbol Digit Modalities Test when compared to controls (Schmitter-Edgecombe & Woo, 2004; Nissley &

Schmitter-Edgecombe, 2002). The current results show no association of hippocampal volume and Trail Making tests A and B and the written component of the SDMT. The verbal component of the SDMT was positively associated with hippocampal volume at both 1 and 6 months post injury suggesting poorer performance with smaller volumes.

Reduced motor speed on the Grooved Pegboard is often related to global outcome after head injury (Clifton et al., 1993). The present study found significant negative associations of hippocampal volume at 1 and 6 months post injury with score on the Grooved Pegboard using the non-dominant hand suggesting that reduced hippocampal volume is associated with increased task completion time.

Limitations

Region of interest measurements have been criticised for being labour-intensive and time consuming as well having inevitable potential inter and intra-observational measurement errors (Ashton et al., 1997; Shen, 2002). However, manual tracing has been found to offer sensitive and specific detection of hippocampal atrophy (Jack, et al., 1989; Cendes et al., 1993) and was used in 90% of protocols for hippocampal volumetry as recently surveyed in a recent review (Geuze, Vermetten & Bremner, 2005).

Analysis of brain structures in relation to head injury has yielded mixed results which may in part be due to methodological differences such as normalising for head size. Normalising includes the use of intracranial volume (Bigler et al., 1997; Tate &

Bigler, 2000) and intracranial area (Juottonen et al., 1999; Kemppainen et al., 2003) either as a ratio measure or as an analysis of covariance approach and the use of different methods may introduce variation.

Other methodological differences which may introduce variability is slice thickness. Although hippocampal volumetry using slice thickness of 1, 3 and 5mm found no significant differences in volume, 'thick' slices are still not recommended as there is an increased possibility of occasional bias in total volume compared to 'thin' slices (Laakso et al., 1997). The method of processing the images, specifically the method of pixel counting, may also contribute as the software used may count the pixels inside the trace, include the row of pixels under the trace in the region of interest or include the row of pixels outside of the trace in the region (Jack et al., 1995).

Perhaps the main methodological disparity is the tracing of the region of interest, especially in the hippocampus. The hippocampus is a structure which has no clear boundaries, unlike the mid-sagittal corpus callosum. Therefore, researchers have used different anatomical boundaries to quantify hippocampal volume. Some studies have measured the hippocampal formation incorporating the hippocampus, alveus and fimbria (Baxendale, Thompson & Kitchen, 2000); the dentate gyrus, hippocampus proper and subicular complex (Laakso et al., 1998) or hippocampus proper, dentate gyrus, subiculum, fimbria and alveus (Hsu et al., 2002; Villarreal et al., 2002).

Different criteria have been used to establish the anterior, posterior and inplane boundaries of the hippocampus itself. Measurements have included the tail and body

but excluded the head (Spencer, McCarthy & Spencer, 1993), the head and body but exclusion of the tail and measurement of the hippocampus along its entire anterioposterior length (Cook et al., 1992; Bilir et al.,1998; Hackert et al., 2002). Further variability is introduced by variations on which slices are chosen to mark the beginning and the end of the hippocampus.

Inconsistencies in neuropsychological methodology may also explain the mixed results found in the literature. For example, studies vary in the use of tests for assessment, time between injury and imaging and/or assessment, injury severity and measurement of injury severity, diagnostic criteria and actual brain pathology such as focal brain lesions and diffuse injury.

Summary and Conclusions

Analysis showed that the right hippocampus was significantly larger than the left hippocampus which has been widely reported in the hippocampal literature. Age effects in the corpus callosum were found to be significant which has also been previously reported. There was no overall volumetric change for the hippocampus and corpus callosum between 1 and 6 months after injury but the results demonstrated that there was hetereogeneity in individual changes. Lack of gross structural changes may be due to the method of volumetry applied in this investigation. Other methods more sensitive to microscopic pathology may have detected physical changes that region-of-interest methodology failed to detect.

Although the association of hippocampal volume with the GCS was modest, significant correlations and the associated trend indicate that the hippocampus is vulnerable in head injury and is especially vulnerable to diffuse injury as measured by the GCS. With regards cognitive testing undertaken at 1 and 6 months after injury, correlations with neuroanatomy displayed a trend whereby associations were stronger at 6 months. Associations reaching statistical significance were established for the hippocampus with immediate and delayed recall at 6 months and with the Grooved Pegboard using the dominant hand at both 1 and 6 months after injury. Thus, hippocampal atrophy was sufficient to have affected memory and visual-motor integration and mental processing speed.

The results of this volumetric analysis suggest that although no gross structural changes occurred between 1 and 6 months post injury, physical hippocampal changes did occur as evidenced by associations with the GCS indicating diffuse damage and by a decline in cognitive performance, the nature of which is also in accordance with diffuse injury to the hippocampus.

CHAPTER 6: VOXEL-BASED MORPHOMETRY

Overview of Voxel-Based Morphometry

In addition to region-of-interest measurements, statistical parametric mapping (SPM) in the form of voxel-based morphometry (VBM) was also used (Ashburner & Friston, 2000). Although VBM has been used to investigate brain structures in normal and diseased states, its use has been limited with regard to head injury. The VBM methodology used in the current investigation is unique whereby the use of whole scan analysis and analysis using masked images has, to date, not been implemented in the study of head injury.

SPM is a voxel based set of methods that allows localisation of structural or functional differences between subject groups on a voxel-by-voxel basis and can be used to analyse various imaging modalities such as MRI, fMRI, PET and SPECT; future releases of the software package will also be able to analyse EEG and MEG. SPM allows the construction and assessment of spatially extended statistical processes used to test hypotheses about neuroimaging data. Most statistical parametric maps are based on linear models such as correlation coefficients and t-tests, which are both special cases of the general linear model, to identify regions of brain tissue with increased or decreased concentration that are significantly related to the particular effects being studied (Friston et al., 1995). VBM is a subset of SPM processes optimised for the analysis of structural images. With VBM, CSF and grey/white matter are extracted from spatially normalised images, smoothed and statistically analysed to localise and make inferences about group differences and/or correlations. Each voxel is individually analysed using a standard univariate

statistical test and the resulting parameters are represented in an image – the statistical parametric map. Voxel-based morphometry is becoming more widespread and has been used to measure structural brain changes in diseases such as Alzheimer's (Frisoni et al., 2005), autism (Salmond et al., 2003), head injury (Gale et al., 2005; Salmond et al., 2005), Schizophrenia (Wilke et al., 2001) and herpes simplex encephalitis (Gitelman et al., 2001).

Spatial Normalisation

In order to compare homologous structures or cortical areas, scans must conform to a known standard anatomical space across all brains under consideration. Spatial normalisation involves the transformation of the subjects' scans into a common stereotaxic space by minimising the sum of squared differences between the subjects' scans (Ashburner & Friston, 2000). The space of the template images is based upon the Talairach atlas system (Talairach & Tournoux, 1988) and includes the cerebellum. The stereotactic space in SPM99 is based on the 152-subject brain template from the Montréal Neurological Institute (Evans et al., 1993). This standard was chosen rather than the MNI305 official standard brain because T2- and proton density weighted images were also available allowing SPM99 to provide a greater range of MR contrasts which can be normalised to the same stereotaxic space. Although the MNI templates have been officially adopted as an international standard by the International Consortium for Brain Mapping (Carmack et al., 2004), they are not atlases and because of this, the neurological community currently consider the Talairach atlas as the standard reference for locating structures and functional areas.

To assure optimal spatial normalisation, the image and the template should be in a similar starting orientation. If the image orientation is particularly misaligned, the normalisation procedure can be helped by first manually aligning the image to the anterior commissure-posterior commissure line. Spatial normalisation consists of two steps: first, the determination of an optimum 12-parameter (translations, rotations, zooms and shears) set of affine transformations from the image to the template and second, a non-linear estimation of deformations that matches the image to the template (Ashburner & Friston, 2000). The first step uses a Bayesian framework where the maximum *a posteriori* estimate of the spatial transformation is made using prior knowledge of the normal variability of brain size whereas the second step accounts for global nonlinear shape differences, which are modeled by a linear combination of smooth spatial basis functions (Ashburner & Friston, 1999). The nonlinear registration involves estimating the coefficients of the basis functions that minimise the residual squared difference between the image and the template, while simultaneously maximising the smoothness of the deformations.

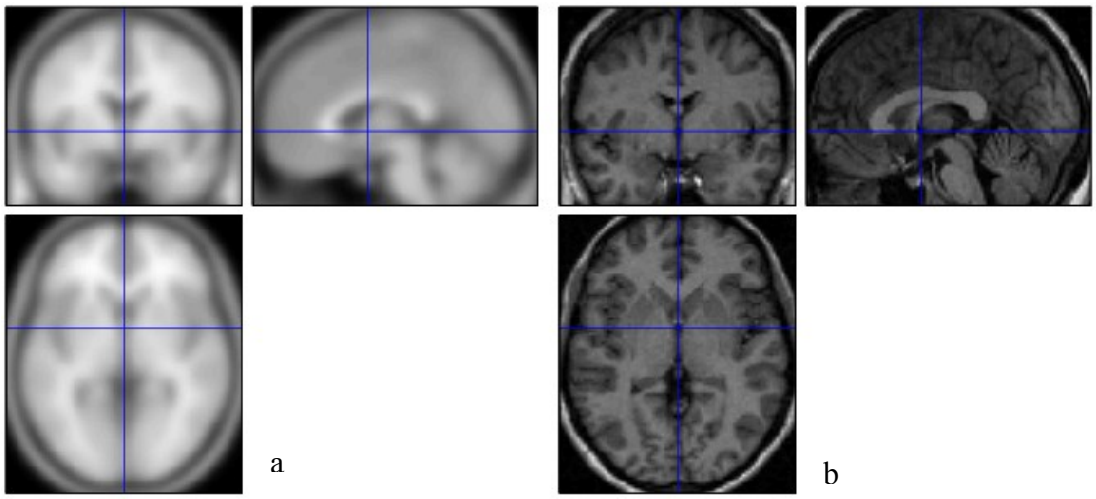


Figure 6.1. Template (a) and spatially normalised brain (b)

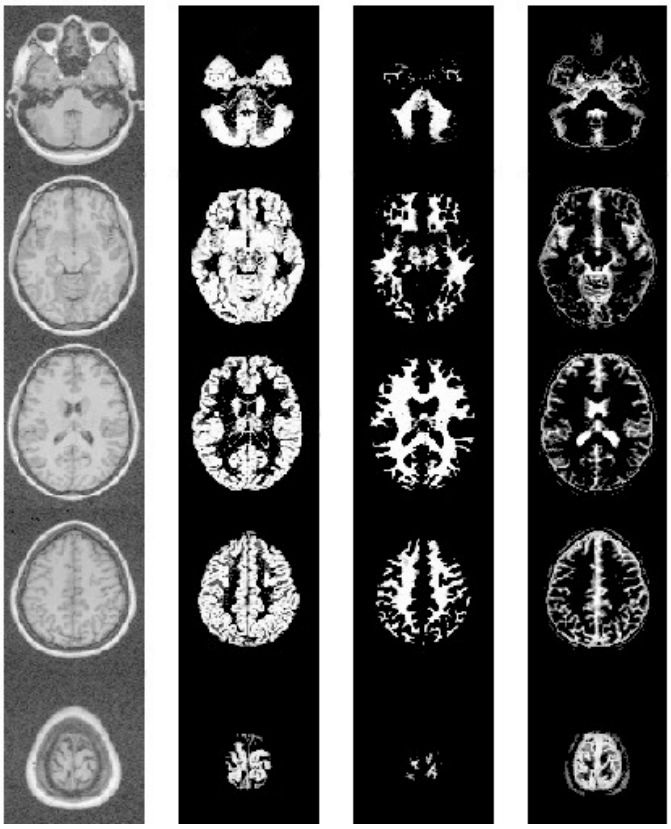
This method of spatial normalisation does not attempt to match every cortical feature precisely, but only corrects for global brain shape differences; for if the spatial normalisation was perfectly exact, then all the segmented images would seem identical and no significant smaller scale changes would be detected between brains. Consequently, VBM detects differences in the regional concentration of grey matter (or white matter or CSF) in brain regions at a local scale having disregarded global brain shape differences (figure 6.1).

Imperfections in spatial normalisation may present themselves as artifacts in particular anatomical locations. The sensitivity of VBM to detect changes may be compromised due to such effects; regions of high variance may lead to a reduced sensitivity of VBM to detect subtle atrophy and increased sensitivity to atrophy in regions of low variance. However, such problems are addressed by the nature of VBM analysis by using a regionally specific estimate of variance (Good et al., 2001).

Segmentation

The segmentation procedure in SPM99 classifies healthy brain tissue into 3 compartments, grey matter, white matter and CSF (figure 6.2), on the basis of intensity values and employs a mixture model cluster analysis technique which assumes that MR images contain different tissue types and each voxel contains only one of these tissue types. Segmentation involves the comparison of the voxel's intensity value with voxels from a set of similarly normalised prior probability maps (providing *a priori* knowledge of tissue types) which specify the likelihood that each voxel belongs to one of the tissue types. The intensities of voxels belonging to each

of these tissue classes conform to a normal distribution; the assignment of voxels to a particular tissue type is determined iteratively depending on the mean and variance of the tissue types for the brain being analysed (Ashburner & Friston, 2000).



a **b** **c**

Figure 6.2. Segmented brain: grey matter (a), white matter (b) and CSF (c)

As with region of interest measurements, partial volume effects where voxels can contain more than one tissue type can prove detrimental to the successful segmentation of data as it causes the distributions of the intensities to deviate from normal; for example, periventricular white matter can be classed as grey matter. Therefore, as this problem is confounded with larger voxel dimensions, it is essential that resolution of images is high, 1mm or 1.5mm isotropic voxels (Ashburner & Friston, 2000). It has been reported that non-brain voxels from dural venous sinuses,

scalp fat and diploic space can be misclassified as grey matter due to having indistinguishable intensity values from grey matter. This can be addressed by removing non-brain voxels by using an automated brain extraction technique prior to normalisation (Good et al., 2001). Successful segmentation results from data being well registered to the prior probability images. As these probability images are based on healthy brains, the segmentation of pathological brains can prove problematic, as they are harder to register. This will be discussed in more detail later.

The segmentation step also incorporates an automatic image intensity nonuniformity correction to address image intensity variations caused by radio frequency inhomogeneity of MR scanning and to address image-density variations caused by different positions of cranial structures within the MRI head coil (Ashburner & Friston, 2000). Although these intensity variations, which vary with pulse sequence, MR field strength and body tissue, have minimal impact on visual inspection of images, they become an important issue when quantitative information of tissue is needed especially when using automatic segmentation algorithms which depend on intensity values. All non-uniformity correction methods involve estimating a smooth function that modulates the image intensities.

VBM and Pathological Data

Problems with spatial normalisation can occur when normalising brains that contain gross anatomical pathology. This pathology can consist of quantitative changes in the amount of a specific tissue (such as cortical atrophy) or qualitative changes in

anatomy involving the addition or removal of normal tissue (such as ischaemic tissue, haematoma and focal lesions).

The algorithms used to optimise linear and non-linear transformations in spatial normalisation are dependent on the intensity values of the data. Lesions have abnormal voxel intensities that can adversely affect the outcome of normalisation as the algorithms attempts to improve the match between the image and the template at the lesion site leads to distorted images due to misregistration. This misregistration has been shown in previous studies (Brett et al., 2001; Gitelman et al., 2001; Fiez, Damasio & Grabowski, 2000).

Such a problem can be addressed by using constraints during the warping process to exclude bias that the lesion may impose on the transformation of undamaged tissue (Ashburner & Friston, 1999). Another method of tackling transformation bias is to use cost-function masking (Brett et al., 2001). Normalisation parameters are determined via the minimisation of a cost function, typically an index of local intensity matching between the template and the image (Ashburner & Friston, 1999). Cost-function masking involves creating a mask of the lesion ensuring that the lesioned area is excluded from the non-linear part of the spatial normalisation (figure 6.3).

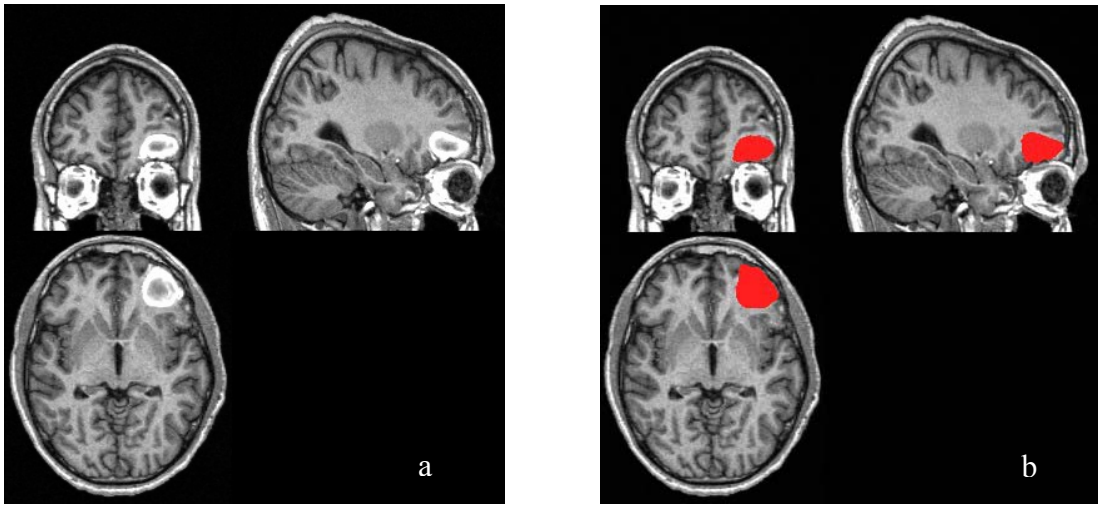


Figure 6.3. MR image showing focal lesion (a) and corresponding lesion mask (b)

As with normalisation, segmentation of the image into its constituent parts (grey matter, white matter and CSF) is dependent on the intensity values of the data. Many algorithms used in segmentation employ a Gaussian model based on global and/or local image intensity statistics for classification of different tissue (Ashburner & Friston, 1997; Grabowski et al., 2000). Consequently, lesioned tissue may produce MR signal intensities that falsely represent other tissue types thereby indicating tissue loss or gain in the lesioned area. Moreover, subsequent smoothing of the segmented images may lead to a considerable underestimation of lesion tissue loss so that tissue loss would be insufficient enough to be detectable by statistical analysis (Mehta et al., 2003). A recent study by Stamatakis and Tyler (2005) compared four commonly used segmentation algorithms and found that none were successful in segmenting large lesions from T1-weighted images. Lesioned T1 MR images were segmented using algorithms from the mixture model cluster algorithm in SPM99, an optimised method used in SPM2 whereby misclassified voxels were removed, Expectation-Maximisation Segmentation (EMS) which is a model-based approach (Van Leemput et al., 1999a, 1999b) and a Markov random field (HMRF) model

(Zhang, Brady & Smith, 2001). Subsequent analysis determined that regions in the image with marked reductions in signal resulted in segmentation errors of white matter misclassified as grey matter using all four algorithms (Stamatakis & Tyler, 2005). To address the issue of segmentation errors when analysing pathological brains, a method of using spatially smoothed, unsegmented images has been proposed and has been shown to be effective in the detection of large lesions in individual patients when compared to a control group (Stamatakis & Tyler, 2005).

Spatial Smoothing

The process of spatial smoothing involves averaging voxels in an image with their neighbour which has the effect of blurring the sharp edges in the smoothed image and is the next of the SPM pre-processing steps. Smoothing converts the image from a high-resolution probabilistic map of grey matter location, into a low-resolution image of the comparative amount of grey matter in the sphere of the smoothing kernel (Gitelman et al., 2001). It is also used to suppress noise and effects due to residual differences in functional and gyral anatomy. Smoothing is critical as it increases signal to noise ratio, rendering the data more normally distributed so that they conform more closely to a Gaussian field model. This is imperative when Gaussian field theory is used to make statistical inferences about regionally specific effects (i.e., assign p-values) as in VBM.

Smoothing in SPM involves three-dimensional convolution of an image with a Gaussian kernel which has the shape of a normal distribution curve. The standard statistical terminology defines the width of the Gaussian curve in terms of sigma but

when it is used for smoothing, the width is described with another related measure, Full Width at Half Maximum (FWHM). In smoothed data, the intensity in each voxel is a locally weighted average of the tissue type concentration from a region of neighbouring voxels. This region is defined by the size of the smoothing kernel which also determines the scale at which anatomical changes are most sensitively detected (Ashburner & Friston, 2000). Thus, if the area under investigation approximates 12mm, a smoothing kernel of 12mm would be implemented.

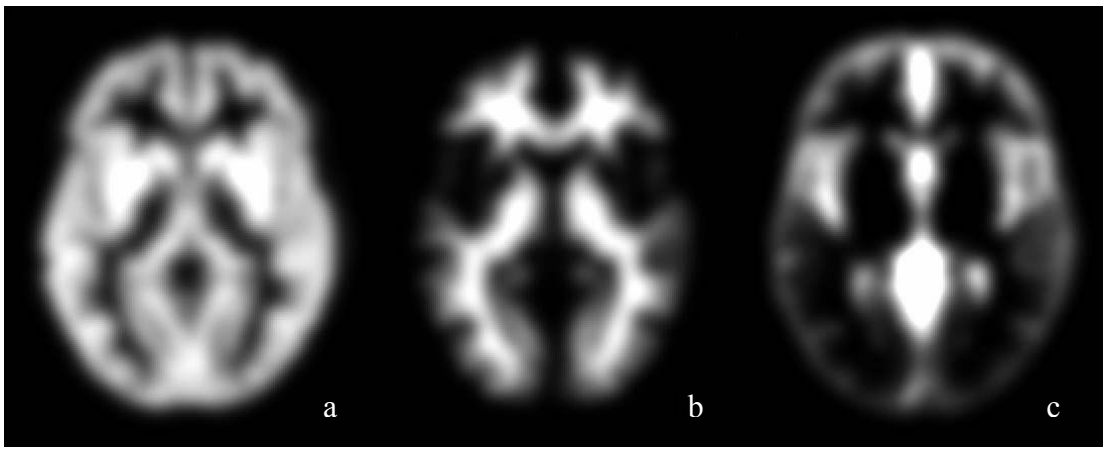


Figure 6.4. Smoothed images of grey matter (a), white matter (b) and CSF (c)

As spatial normalisation can result in slight imperfections such as individual differences in sulcal and gyral anatomy, smoothing data corrects for these by allowing neighbouring voxels to share more information. It also ensures that effects between different patients are assessed on a reasonable spatial scale with respect to functional anatomy (Ashburner & Friston, 2000). It is important to select the correct size of kernel for the anatomical area under investigation as too little smoothing can result in increased image noise and can disrupt assumptions of normality while too much smoothing can reduce statistical significance by reducing specificity of anatomical regions (Stamatakis, Wilson & Wyper, 2000).

Smoothing makes the subsequent voxel-by-voxel analysis more comparable to a region of interest approach, because each voxel in the smoothed images contains the average concentration of the gray matter from within the selected voxel and, to a lesser extent, from neighboring voxels (the smoothed volume can be thought of as a weighted region of interest). This is sometimes referred to as grey matter density but should not be mistaken for cytoarchitectonically measured cell packing density (Ashburner & Friston, 2000).

Alternative Approaches

Pre-processing steps of normalisation, segmentation and smoothing are not exclusive to SPM but can be employed by other methods such as regional analysis of volumes examined in normalised space (RAVENS) (Davatzikos et al., 2001). The main difference between RAVENS and SPM is in the spatial normalisation method; while SPM uses linear and non-linear warping algorithms, RAVENS uses a high-dimensional elastic transformation. The methods also differ in the way in which the spatial distribution of grey matter, white matter and CSF are determined in the stereotactic space. SPM removes global differences with the implementation of affine transformations whereas RAVENS preserves the volumes of the tissue classes at the local and global level during spatial normalisation (Davatzikos et al., 2001).

As well as the standard VBM procedure, there has since been developed an 'optimised' method (Good et al., 2001). In addition to incorporating a fully automatic brain extraction technique to remove skull, scalp tissue and dural venous sinus voxels, the optimised method involves creating study-specific tissue templates

that are used for normalisation rather than using the MNI template applied in standard VBM. This is achieved by segmenting the MR images in their native space into the different tissue classes then normalising these to stereotactic space using corresponding tissue templates. The normalisation parameters derived from these transformations are then applied to the wholebrain images in native space before undergoing segmentation again. These extra steps can reduce misclassification errors which may occur where patient groups have significantly larger ventricles leading to an apparent increase in grey matter due to normalisation parameters failing to distinguish between the ventricles and surrounding grey matter (Mechelli et al., 2005). The optimised method also introduces an optional modulation step that incorporates volume change during normalisation allowing assessment of tissue volume as well as tissue concentration. This step involves the multiplication of the normalised segmented tissue class by its relative volume before and after normalisation to allow for detection of absolute tissue volume (Good et al., 2001).

All of the aforementioned approaches to VBM are valid and the use of one over the other is dependent on the area under investigation and the questions being asked (Mechelli et al., 2005).

Voxel-Based Morphometry Methodology

Standard VBM image analysis was performed using SPM99 software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK; http//www.fil.ion.ucl.ac.uk/spm) running in Matlab 5.0 (MathWorks Inc., Natick, MA, USA) on a SPARC 10 workstation (Sun Microsystems, Mountain View, CA,

USA). This image analysis technique was used to detect hippocampal changes between 47 head-injured patients and 18 control subjects as well as changes between the head-injured patients' scans at 1 and 6 months after injury.

MR Image Acquisition

The MR images used in VBM analysis were the same data set used for region-of-interest measurements and full MR image acquisition details have been described elsewhere (Chapter 4). Briefly, T1-weighted images were acquired using a 3D gradient echo sequence, MP_RAGE: flip angle of 12°, a field view of 250mm and a matrix size of 256 x 256 resulting in 256 slices with a voxel size of 1.4 x 1 x 1mm^3. A pre-processing step such as that used in Analyze to transform the voxels into cubic dimensions was not necessary as the process of spatial normalisation contends with this matter.

Control Group

Recruitment of 18 age-matched control subjects was necessary for comparison with the patient group and was approved by the medical ethics committee of the Southern General Hospital. Subjects were recruited from the Southern General Hospital, the University of Stirling and via advertisements placed throughout the university campus. Exclusion criteria for VBM control subjects were the same as for the patient group namely; age (under 16 or over 75 years), mental handicap, epilepsy, residence distance from SGH (>60 miles), prior intracranial operation, prior head injury requiring neurosurgery, previous hospital treatment for psychiatric illness,

prior hospital treatment for alcoholism or drug abuse and dependency due to brain disease pre-injury. Also, due to the inclusion of an MRI scan, each patient was screened for exclusion criteria for MRI i.e. possession of electronic devices/implants, any metallic fragments in the eyes or other contra-indications for MRI. All MR data was acquired using the same protocol as the patient group.

Two subjects, both aged 67, were initially recruited to the study but after visual examination of their MRI scans, it was revealed that both had noticeable ventricular enlargement consistent with mild atrophy. Consequently, they were eliminated from the study and reports forwarded to their GPs. Another subject was also eliminated due to a scanning error that produced an unusable image.

Table 6.1. Age Categories of Control Group

Age-Group	N	%
16-19	2	11
20-29	11	61
30-39	3	17
40-49	2	11

The mean age of the control group was 41.1 years (SD 13.8, range 18 – 42) and the gender ratio was 12 males: 6 females. A paired samples t-test showed that there were no age differences between groups, $t(17) = 1.2$, $p = 0.25$.

Spatial Normalisation

Constraints were determined for parameter estimation to normalise images to the T1 template. For patient images, the parameters used were 8 x 8 x 8 non-linear basis functions, 16 non-linear iterations and heavy regularisation. Regularisation stabilises

deformation estimations by penalising large deformations. If spatial normalisation results in excessively warped images, heavy regularisation is required whereas decreased regularisation will be required where images do not get warped enough in order to match the template. Control images required a fewer number of non-linear basis functions and less regularisation. Thus, 7 x 8 x 7 non-linear basis functions, 16 non-linear iterations and medium regularisation were used. The normalised images were resampled to 1 x 1 x 1mm voxel size and the template bounding box used voxel dimensions of -90:90 -126:90 -72:108 mm^3.

Successful spatial normalisation was determined by visual comparison of homologous regions of the template and normalised images using the 'check registration' option in SPM99; this function allows simultaneous visual inspection of anatomical regions on 2 or more images. The anterior commissure was identified in each image and particular attention was paid to the correct normalisation of the temporal lobes and hippocampus as a hippocampal mask was applied to the spatially normalised, smoothed images. Of the 47 images at 1 month post injury, 6 did not normalise correctly due the introduction of distortions resulting from the pathological states of the 6 brains i.e. large lesions and/or large haematomas. For example, ventricles and cortex were misaligned when compared to the template. Also, 5 images at 6 months post injury also required masking due to areas of malacia. To resolve this, the pathology was masked to prevent normalisation from performing non-linear transformations in these areas (Brett et al., 2001). Lesion and haematoma masks were created using manual tracing in the MRIcro brain image display software (Rorden, 2002; http://www. psychology.nottingham.ac.uk/staff/cr1/mricro.html). The resulting region of interest was then converted from a compressed format to the

uncompressed Analyze format that is compatible with SPM99. Various permutations of the available parameters were tested in order to determine optimal spatial normalisation (table 6.2).

Table 6.2. VBM Parameters of Masked Images

Non-Linear Basis Functions	Non-Linear Iterations	Regularisation
8 x 8 x 8	16	Medium
8 x 8 x 8	16	Heavy
8 x 8 x 8	16	Medium
4 x 4 x 4	8	Medium
7 x 8 x 7	16	Medium
8 x 8 x 8	16	Medium

Segmentation

All normalised images were segmented into grey matter, white matter and CSF based on voxel intensities and *a priori* knowledge of brain tissue distribution using the 152 MNI template. SPM was directed to correct for intensity inhomogeneities. Images were visually checked in each orientation (axial, coronal and sagittal) using the 'check registration' option in SPM99 to ensure correct segmentation of the individual tissue compartments. Particular consideration was given to the temporal lobes and hippocampus, as the hippocampus was to be isolated from the rest of the brain and analysed separately with neuropsychological data. Previously, each individual image had been visually inspected for the presence and location of lesions and haematomas. None of the patients had large lesions and/or haematomas at or near the hippocampal complex. Therefore, the hippocampi were successfully normalised and segmented into component tissue parts.

Spatial Smoothing

Segmented images were three-dimensionally smoothed using an isotropic Gaussian kernel with full-width half-maximum of 10mm. Most studies using VBM to investigate hippocampal changes have used kernels varying in size from 8mm – 12mm. Reductions in hippocampal grey matter in Alzheimer's patients have been found using an 8mm kernel (Rombouts et al., 2000) and a 12mm kernel (Testa et al., 2004) while 10mm kernels have been used to investigate age effects in the hippocampus (Maguire & Firth, 2003), increased hippocampal grey matter with navigational expertise (Maguire et al. 2003) and hippocampal atrophy in temporal lobe epilepsy (Keller et al., 2002; Keller et al., 2004). Smoothing of the segmented images involved convolution with a 10mm FWHM isotropic Gaussian kernel.

Hippocampal Analysis

As the main focus of VBM analysis was to investigate hippocampal data, images were masked for brain tissue outwith the hippocampi. The bilateral hippocampal mask (figure 6.5) had been previously manually drawn on a spatially normalised healthy brain image using MRIcro (Rorden, 2002; http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html) by Dr E. A. Stamatakis (Department of Experimental Psychology, University of Cambridge, UK) and reused with kind permission. The mask was smoothed to accommodate for interindividual differences allowing all of the hippocampus to be included and was applied to the images after spatial smoothing.

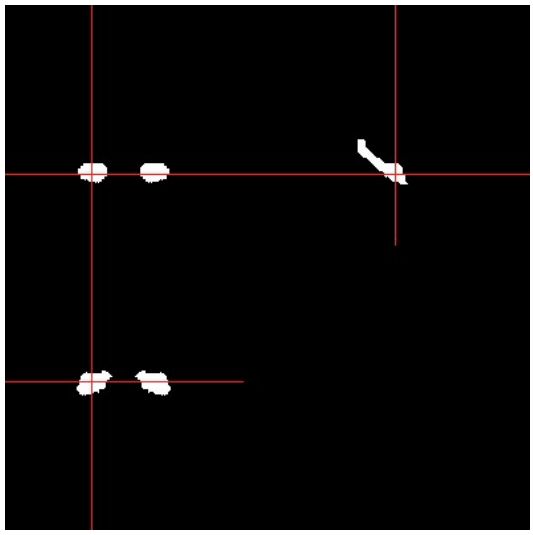


Figure 6.5. Spatially normalised hippocampal mask

Whole Scan Analysis

To assess structural change across tissue types and investigate neuropathology, whole scan analysis was performed on unsegmented images. This method investigated tissue contrast (signal differences) and involved normalising using 8 x 8 x 8 non-linear basis functions, 16 non-linear iterations and heavy regularisation. Instead of segmenting the images into separate tissue compartments, images were skull-stripped by masking the images with the brain mask available in SPM99 and smoothed using a 10mm isotropic Gaussian kernel at full-width half-maximum (Stamatakis & Tyler, 2005; Tyler, Marslen-Wilson & Stamatakis, 2005a). This size of kernel has been shown to be the most reliable for lesion detection and provides minimal false positive and false negative rates (Stamatakis & Tyler, 2003; Stamatakis & Tyler, 2005). Larger smoothing kernels can eliminate lesions, especially small lesions, as they prompt the lesions to merge with the background signal causing them to be indistinguishable and consequently undetectable by VBM analysis.

Statistical Analysis

Voxel-wise statistical analysis performed on spatially normalised segmented images and whole scan images results in statistical parametric maps showing areas where tissue concentration (segmented images) and signal intensity (whole scan images) differs significantly between groups. Differences in tissue concentration that are related to the effects under study are identified using the general linear model (Friston et al., 1995). This allows the use of different statistical tests for purposes such as comparing groups and correlations with covariates of interest. Hypotheses are tested using standard statistical parametric tests, t-tests and F-tests. After applying the general linear model, differences in tissue concentration/signal intensity are tested for significance using the theory of Gaussian random fields (Worsley et al., 1996) which corrects for multiple comparisons as the resulting statistical parametric maps contain the results of many statistical tests (Ashburner & Friston, 2000). The statistical parametric maps are displayed on a 'glass brain' allowing visualisation of clusters in 3 orthogonal planes – axial, coronal and sagittal.

Whole scan analysis involved investigating regionally specific group differences between the patient group and control group and between the patient group at 1 month and 6 months post injury. The distribution of differences in signal intensity across the whole brain was assessed on a voxel by voxel basis and significance was assessed using an uncorrected height threshold of $p<0.001$, $p<0.01$ and $p<0.05$. The next step involved correcting for multiple comparisons over the whole brain at the cluster level. Voxel-level tests identify individual significant voxels whereas cluster level analysis identifies the spatial extent of significant contiguous voxels (Friston et

al., 1995). For each height threshold, inferences were centered on differences that achieved significance at $p<0.05$ after cluster correction.

For analysis of segmented images, concentrations of grey matter, white matter and CSF were analysed separately. Tissue class concentrations were investigated using group analyses between the patient and control group and between the patient group at 1 and 6 months post injury. Tissue class concentrations were also correlated individually with neuropsychological test scores using two linear contrasts (positive or negative correlation). For all analyses, significance was assessed using uncorrected height thresholds of $p<0.001$, $p<0.01$ and $p<0.05$ and reported at $p<0.05$ corrected for multiple comparisons for consistency. This threshold has previously been used in VBM analysis (Mechelli et al., 2005; Keller et al., 2004; Gitelman et al., 2001). For analyses where *a priori* hypotheses can be applied i.e. correlation of hippocampal tissue class with age and with psychological tests involving memory, significance can be analysed at an uncorrected height threshold of $p<0.05$ and clusters not corrected for multiple comparisons (Friston 1997; Keller et al., 2002). However, due to the exploratory nature of the analysis, in all VBM analyses with segmented images significance was reported at an uncorrected height threshold of $p<0.05$, corrected for multiple comparisons at the cluster level and inferences centered on differences that achieved significance at $p<0.05$ after correction for multiple comparisons.

Transformation of MNI Coordinates

During normalisation, SPM99 uses the MNI152 template brain while the Talairach coordinate system is the standard reference system for reporting brain regions. Due to differences in brain shape and size between the two templates, it is necessary to transform MNI coordinates to Talairach coordinates. Approaches for transformation include the Talairach Method of Piecewise Linear Scaling, template-matching using linear affine transformation and the Matlab script, mni2tal, devised by Matthew Brett of the MRC Cognition and Brain Sciences Unit, Cambridge, UK (Brett, 1999; http://www.mrc-cbu.cam.ac.uk/Imaging/Common/mnispace.shtml). A study which investigated these methods found patterns of uncertainty with each method and advised reporting MNI coordinates alongside Talairach regions (Chau & McIntosh, 2005). The present study used the commonly used Matlab mni2tal script to map the MNI coordinates to Talairach coordinates. This approach is based on linear transformations in the regions above and below the anterior commissure providing an individual Talairach coordinate for each MNI coordinate. This method has been used in various VBM analyses such as investigation of grey matter density in schizophrenics (Salgado-Pineda et al., 2003; Job et al., 2002), grey matter density in Broca's area of musicians (Sluming, 2002) and grey and white matter differences in Down Syndrome adults (White, Alkire & Haier, 2003).

Identifying Talairach Regions

Defining Talairach regions can be done by using an automated database known as the Talairach Daemon (http://ric.uthscsa.edu/projects/talairachdaemon.html) which provides anatomical labels for inputted Talairach coordinates (Lancaster et al., 1997;

Lancaster et al., 2000) and manually checked using the Talairach atlas (Talairach & Tournoux, 1988). The Daemon uses a five-level hierarchy to identify and label coordinates. Level one consists of six components; left and right cerebrum, left and right cerebellum and left and right brainstem. Level two divides level one components into their constituent lobes as well as dividing the brainstem into midbrain, pons and medulla. Level three separates each lobe into gyri or gyral equivalents (such as thalamus and striatum) while level 4 is concerned with tissue type whereby level three gyri (or gyral equivalents) are segmented into grey matter, white matter and CSF. Level five of the Talairach daemon hierarchy is concerned with cell population i.e. Brodmann's areas are used to label cerebral cortex and subnuclei are used to label nuclear groups.

CHAPTER 7: VOXEL-BASED MORPHOMETRY RESULTS

1. Whole Scan Morphological Analyses

Regional group differences in signal change were tested using spatially normalised unsegmented images. Proportional scaling in SPM99 was used to remove global signal intensity across subjects whereby the intensity of each voxel was normalised relative to the total intensity of the brain (Kim et al., 2001). The distribution of differences in signal intensity across the whole brain was assessed on a voxel by voxel basis and significance was assessed using an uncorrected height threshold of $p < 0.001$ (unless stated otherwise), corrected for multiple comparisons over the whole brain at the cluster level; inferences were centered on differences that achieved significance at $p < 0.05$ cluster level after correction. Decreases in signal were investigated between the patient group at 1 and 6 months post injury, between the patient ($n = 47$) and control groups ($n = 18$) and for subgroups of patients categorised by cause of injury.

Gender Considerations

Gender differences in brain structures have been demonstrated using VBM (Luders et al., 2004; Good et al., 2001), which prompted a study investigating musicians to use only male subjects (Sluming et al., 2002). However, it is necessary to be careful when extrapolating results of single gender studies to the general population. As the current patient group had a gender imbalance with a male-female ratio of 43:4, it was not practical to include gender as a confounding covariate because of the small number of female cases.

Age as a Covariate of Interest

Numerous studies have investigated the effects of aging in the brain. During the first 6 decades of life, grey matter volume in most brain regions decreases more rapidly followed by a slowed decline in further years (Sowell et al., 2003). A voxel-based study using a large number (465) of normal adult brains found increased loss in frontal and parietal regions and a linear decrease in global grey matter with age although limbic structures (hippocampus, entorhinal cortex and amygdala) were found to have relative grey matter preservation (Good et al., 2001). Grey matter in limbic structures has also been found to be relatively preserved across eight decades (Grieve et al., 2005) and age effects have also been found with regard to personality where larger grey matter volumes have been indicated in self-transcendence personalities in later life (Kaasinen et al., 2005).

As the current patient group encompassed a wide age range (16-66), it was necessary to conduct an analysis to verify any age effects that would have had a confounding affect on subsequent analyses. The resulting statistical parametric maps (figure 7.1) show clusters surviving multiple correction in negative correlational analysis. Thus, these age effects were controlled for in all other analyses by including age as a confounding covariate in the design matrix.

Age was shown to be correlated negatively with signal in the patient group with signal reduction established in three large clusters surviving correction for multiple comparisons at $p < 0.05$. Clusters were located in the left pyramis of the cerebellum, the genu of the corpus callosum and in sub-gyral white matter in the right temporal lobe (figure 7.1).

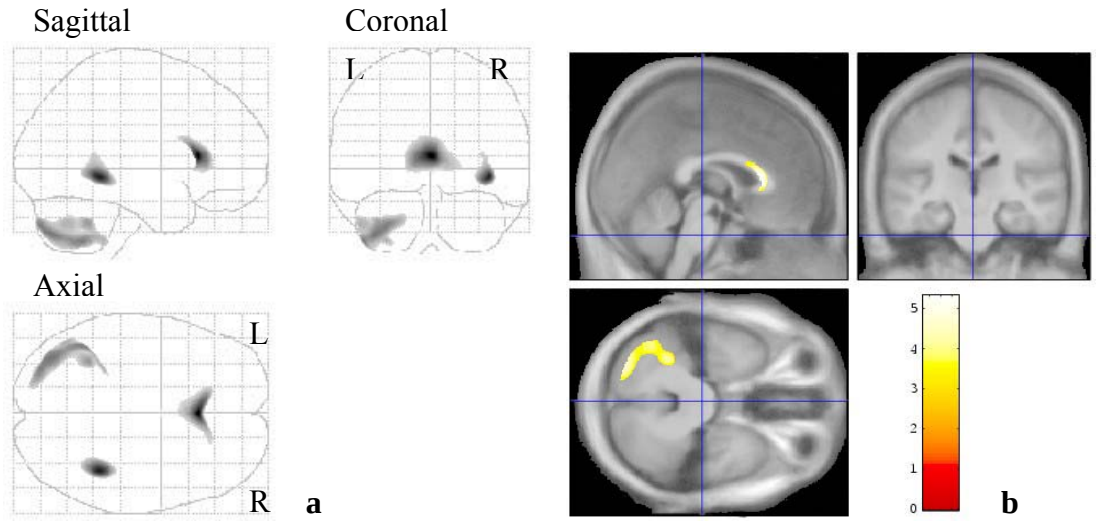


Figure 7.1. SPM superimposed on a 'glass brain' showing voxel clusters negatively correlated with age in the patient group (a) and overlay of SPM on a structural image made from the mean of normalised patient brain images (b)

The largest region of signal reduction was located unilaterally in the left pyramis of vermis of the cerebellum and contained almost twice the amount of voxels as the region located in the genu of the corpus callosum. The smallest region of signal reduction was found in sub-gyral white matter in the temporal lobe.

Table 7.1. Age as a Covariate of Interest in Patient Group at 1 Month Post injury (Height Threshold = $p<0.001$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm)			Peak Z Score
		x	y	z	
6533	L Pyramis	-37	-74	-44	4.17
3699	Corpus callosum	1	25	9	4.65
2287	R Sub-gyrus	40	-42	-5	4.58

Whole Scan Signal Change between Patient and Control Groups

Exploratory analysis was performed to investigate decreases in signal change between the control group and patient group at 1 and 6 months post injury. Clusters of decreased signal were unilaterally located in the left hemisphere in the anterior

cingulate and superior frontal gyrus at 1-month post injury and in the superior frontal gyrus at 6 months post injury indicating poorer brain integrity in the patient group.

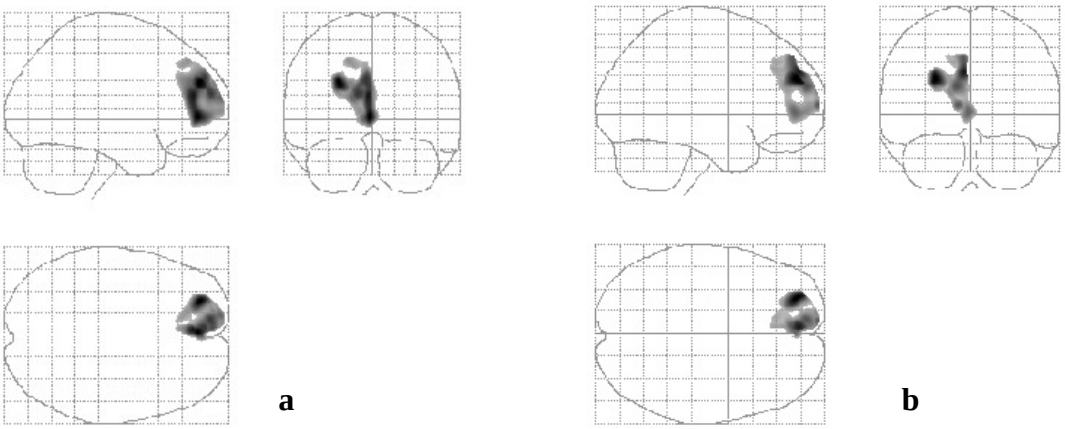


Figure 7.2 Decreases in signal at 1 month (a) and 6 months (b) post injury compared with controls

When analysis was performed using the patient group at 6 months, no clusters at height threshold $p < 0.001$ survived correction for multiple comparisons. At $p < 0.01$, a large cluster of 10164 voxels with the peak voxel in the left superior frontal gyrus was found. This voxel was also significant in the large cluster produced at 1 month which was also performed at threshold $p < 0.01$ for comparison. The statistical map (figure 7.2) indicates that a decrease in signal from the patient group at 1 month to the control group was found in the left frontal lobe, which produced a large cluster (14253 voxels); the peak coordinate of which was located in the left anterior cingulate. Results at 1 month were also significant at the higher threshold of $p < 0.001$.

Table 7.2. Atrophied Regions in Patients at 1 and 6 Months Compared with Controls (Height Threshold = p<0.01; Clusters Corrected for Multiple Comparisons, p<0.05)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
1 month					
14253	L Anterior cingulate	-2	48	2	4.32
	L Superior frontal gyrus	-5	53	32	3.76
6 months					
10164	L Superior frontal gyrus	-5	53	27	4.01

Whole Scan Signal Change between Patients at 1 and 6 Months Post injury

Decreases in signal were investigated between the patient group at 1 and 6 months post injury.

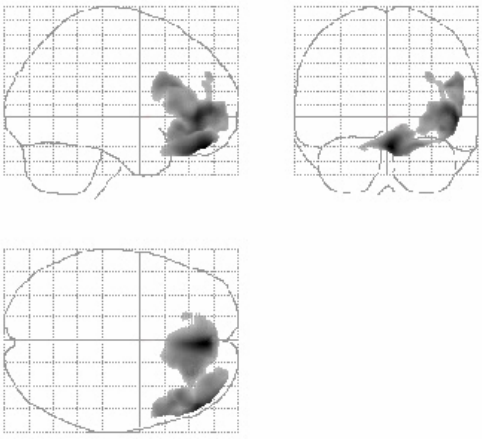


Figure 7.3. Decreases in signal in the patient group between 1 and 6 months post injury

An extensive cluster of 27111 voxels with the peak voxel in the right orbital gyrus was detected at a lower height threshold of $p < 0.05$ after no clusters survived correction for multiple comparisons at $p < 0.001$ or $p < 0.01$. Due to the large size of the cluster, a sample of coordinates was examined on the Talairach & Tournoux atlas to establish the extent of the cluster. Although the peak voxel was located in the

right orbital gyrus, the cluster extended into the right inferior, superior and medial frontal gyri and into the left inferior frontal gyrus.

Table 7.3. Whole Scan Atrophy in Patients Between 1 and 6 Months Post Injury (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm)			Peak Z Score
		x	y	z	
27111	R Orbital gyrus	3	48	-23	4.31
	R Inferior frontal gyrus	51	43	0	3.09
	R Superior frontal gyrus	23	39	-20	2.38
	R Medial frontal gyrus	12	29	-13	2.23
	L Inferior frontal gyrus	-19	33	-21	1.77

Cause of Injury and Patterns in Brain Damage

To examine global morphology in relation to cause of injury, the patient group was subdivided into assault victims and those who had suffered head injury as the result of a fall and compared with the control group (table 7.4).

Table 7.4. Subgroup Statistics

Group	N	Mean Age	SD	Lesions	Haematomas	Both
Falls	17	37.5	14.4	11	12	7
Assaults	16	31.5	12.1	5	6	3
Control	18	41.1	13.8	0	0	0

Assault as Cause of Injury

Figure 7.4 illustrates reduction in signal in the patient group in comparison to controls. At 1 month, a cluster containing 6359 voxels was found in the left frontal lobe with the peak voxel located in the left superior frontal gyrus. A smaller cluster consisting of 2273 voxels was located in the tuber of vermis of the right cerebellum.

This smaller cluster was not evident at 6 months although the cluster in the frontal lobe was still present. Although reduced in size with 1845 voxels, the peak voxel of the cluster remained the same indicating the location of the left superior frontal gyrus.

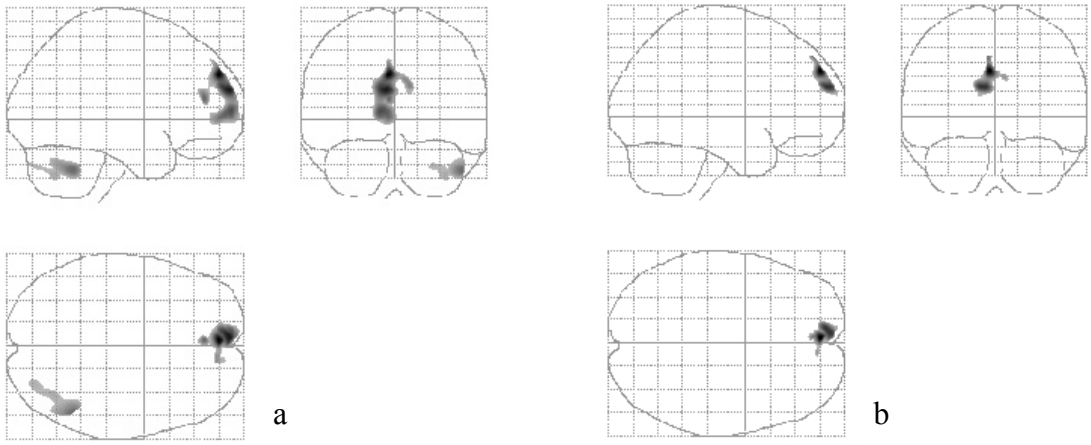


Figure 7.4. Decreases in signal change between the assault patient group at 1 month (a) and 6 months (b) post injury and the control group

Table 7.5. Atrophic Changes Between Control Group and Assault Victims (Height Threshold = $p<0.001$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
1 Month					
6359	L Superior frontal gyrus	-4	54	33	5.65
2273	R Tuber	49	-52	-36	4.34
6 Months					
1845	L Superior frontal gyrus	-4	54	33	4.79

Analysis comparing signal change of the assault subgroup at 1 and 6 months post injury yielded no decreases at height thresholds $p < 0.001$, $p < 0.01$ or $p < 0.05$ indicating that the difference in cluster size in the left superior frontal gyrus was not great enough to be detected.

Falls as Cause of Injury

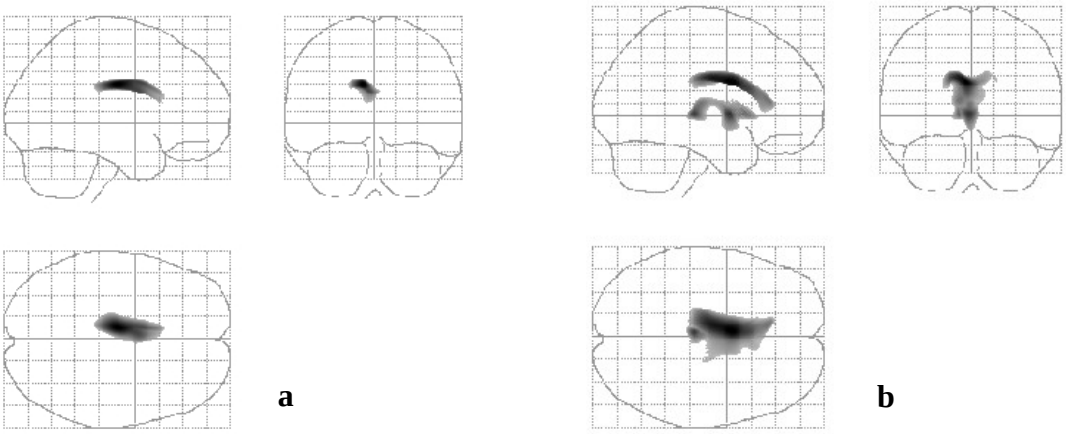


Figure 7.5. Decreases in signal change between the falls patient group at 1 month (a) and 6 months (b) post injury and the control group

Signal decrease in the falls subgroup in relation to controls was evident in the left hemisphere with peak voxels located in the cingulate gyrus at 1 month and in the left cingulate gyrus and left lateral ventricle at 6 months (figure 7.5). The cluster in the cingulate gyrus increased in size from 3862 voxels at 1 month post injury to 7556 voxels at 6 month post injury (table 7.6). At 6 months, a cluster was also located in the ventricles with a peak voxel situated in the left lateral ventricle. These results suggest that the decrease in signal from the control group to the patient group is due to brain atrophy caused by head injury.

Table 7.6. Atrophic Changes between Control Group and Fall Patients (Height Threshold = $p<0.001$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
1 Month					
3862	L Cingulate gyrus	-8	-13	30	4.42
6 Months					
7556	L Cingulate gyrus	-4	3	28	4.78
3518	L Lateral ventricle	-2	2	3	4.33

Analysis comparing signal change between the fall subgroup at 1 and 6 months post injury yielded no decreases at height thresholds $p<0.001$, $p<0.01$ or $p<0.05$. Thus, there was not enough decrease in signal between the patient group at 1 and 6 months for VBM to detect any changes.

2. Analyses Using Segmented MR Images

T1-weighted images were segmented into grey and white matter and CSF and then masked using a smoothed mask constructed in MRIcro, to exclude all brain except hippocampi. Thus, concentrations of grey matter, white matter and CSF were analysed separately and correlated individually with the neuropsychological test scores. For all analyses, significance was initially assessed using uncorrected height thresholds of $p < 0.001$, $p < 0.01$ and $p < 0.05$. For analyses with no *a priori* information, significance is reported at an uncorrected height threshold of $p < 0.05$, corrected for multiple comparisons at the cluster level and inferences centered on differences that achieved significance at $p < 0.05$ after error correction. For analyses where there were *a priori* hypotheses i.e. correlation of hippocampal tissue class with age and with psychological tests involving memory, significance was initially analysed at an uncorrected height threshold of $p < 0.05$ and clusters were not corrected for multiple comparisons (Friston 1997; Keller et al., 2002). Consequent significant clusters were then checked to establish if they survived correction for multiple comparisons and reported as such for consistency. The peak voxel for each cluster is reported.

Morphological Comparison of Segmented Images

Age as a Confounding Covariate

There is conflicting evidence regarding the effects of age on the hippocampus. Although limbic structures such as the hippocampus have been shown to have relative grey matter preservation (Grieve et al., 2005; Good et al., 2001), age-related effects have been detected in hippocampi with regard to autobiographical memory (Maguire & Frith, 2003), which posits how normal aging affects the hippocampus. As the current patient group was not age-matched with a range of 16-66, it was necessary to conduct an analysis to verify any age effects that would have had a detrimental affect on subsequent hippocampal analyses.

Correlations of Age with the Patient Group at 1 Month

The statistical parametric map (figure 7.6) shows clusters surviving correction for multiple comparisons ($p<0.05$) in negative correlation with grey and white matter segments and in positive correlation with CSF. Bilateral grey matter clusters were found in the left hippocampal gyrus and in the right thalamus. Findings of clusters in areas that may not be expected when masking images to include only the hippocampus can be explained by the effect of smoothing the images and smoothing of the mask. Due to the size of the smoothing kernel used, results outside of the hippocampus were to be expected.

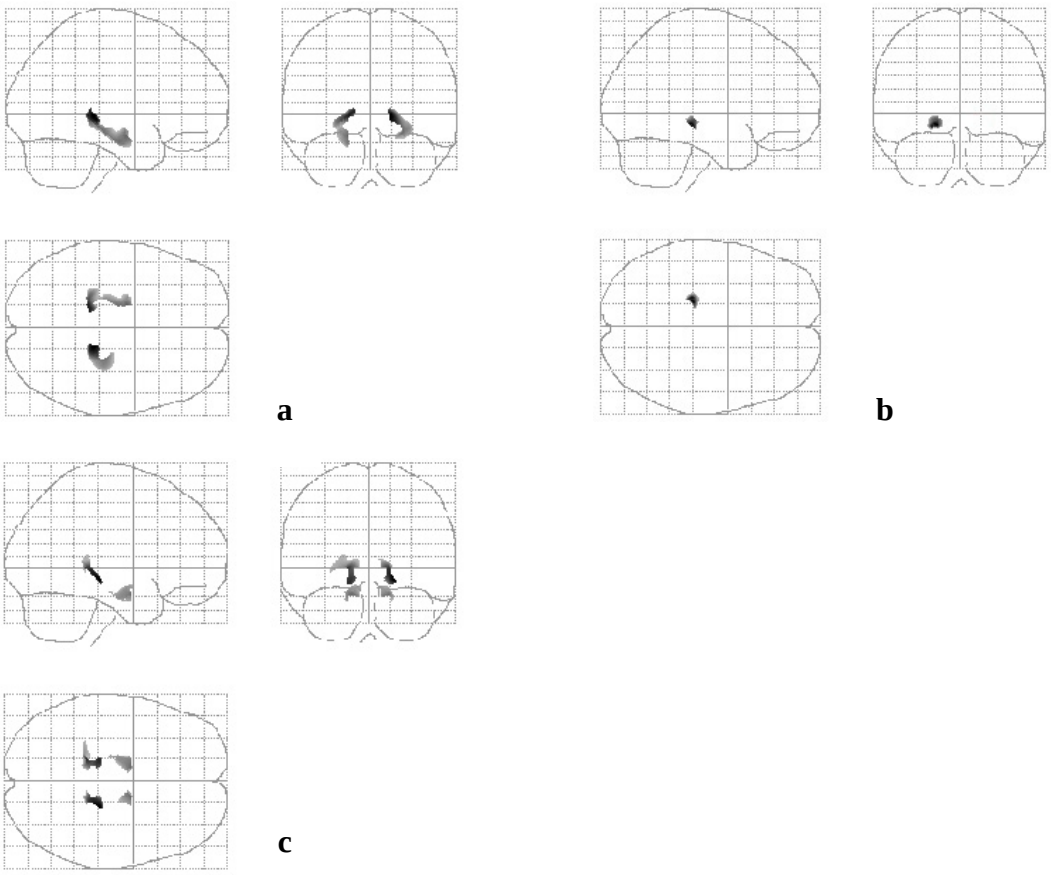


Figure 7.6. Negative correlations of grey matter (a), white matter (b) and positive correlation of CSF (c) with age and patient group at 1 month

A unilateral white matter cluster, the smallest cluster from the three tissue classes, was located in left parahippocampal gyrus. Bilateral clusters of CSF were distributed in the left and right limbic lobes with the left lobe providing two clusters which were larger than the two corresponding clusters in the right lobe. The white matter peak coordinate in the parahippocampal gyrus was found to be significant at a higher threshold of $p < 0.01$ and CSF clusters in the limbic lobe were found to be significant at height thresholds of $p < 0.01$ and/or $p < 0.001$ indicating the strength of correlation between these regions and age.

Correlations of Age with the Patient Group at 6 Months

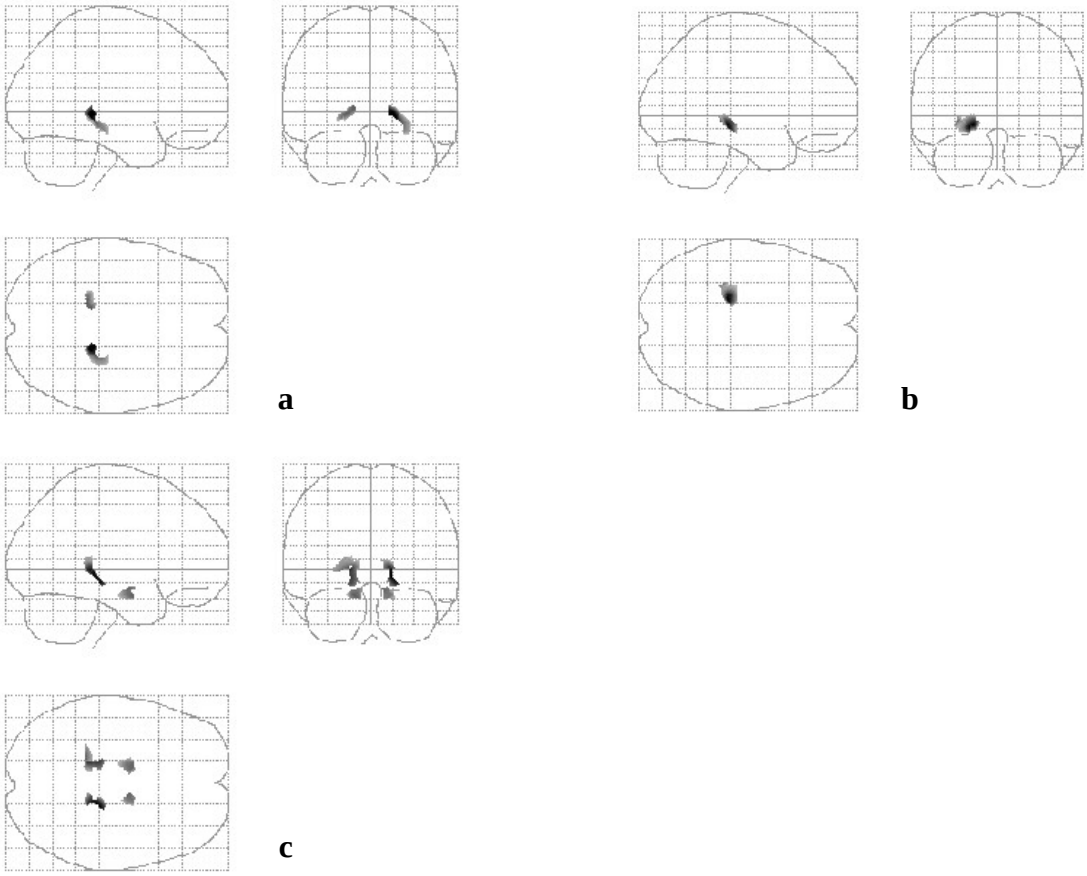


Figure 7.7. Negative correlations of grey matter (a), white matter (b) and positive correlation of CSF (c) with age and patient group at 6 months

At 6 months, grey matter concentration was negatively associated with age with bilateral clusters in the left and right parahippocampal gyri consisting of 340 and 627 voxels respectively. White matter concentration was also negatively associated with age represented by a unilateral sub-gyral cluster of 812 voxels. CSF concentration was positively associated with age with bilateral clusters in the left and right parahippocampal regions. These results mirror the correlations of age at 1 month where age was also negatively associated with grey and white matter concentration and positively associated with CSF concentration.

Table 7.7. Correlations of Age and Segmented Hippocampal Images (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
1 Month					
1336	R Thalamic GM	15	-32	-1	4.35**
1009	L Parahippocampal gyral GM	-16	-37	-1	3.97**
337	L Parahippocampal gyral WM	-22	-27	-11	2.83*
569	L limbic lobe CSF	-15	-28	-10	4.06**
547	L limbic lobe CSF	-11	-2	-21	3.17*
371	R limbic lobe CSF	21	-27	-9	4.69**
344	R limbic lobe CSF	13	-2	-21	3.39*
6 Months					
627	R Parahippocampal gyral GM	16	-35	-3	4.96**
340	L Parahippocampal gyral GM	-16	-38	0	3.16*
812	L Sub-gyral WM	-20	-30	-7	3.20*
713	L Parahippocampal gyral CSF	-16	-32	-7	3.68**
438	L Parahippocampal gyral CSF	-13	-2	-20	2.92*
312	R Parahippocampal gyral CSF	17	-32	-6	4.22**
279	R Parahippocampal gyral CSF	13	-2	-19	2.96*

GM – Grey Matter; WM – White Matter
*Significant at $p<0.01$ height threshold, **Significant at $p<0.001$ height threshold

These age effects suggest that an increase in age is associated with a decrease in grey and white matter concentration and a corresponding increase in CSF concentration. Therefore, these age effects were controlled for in all other analyses by including age as a confounding covariate in the design matrix.

Hippocampal Differences between Patient Group at 1-Month and Controls

Contrasts were defined to assess differences in grey matter, white matter and CSF concentration in the hippocampal region between the patient group (n = 47) and the control group (n = 18). Contrasts referred to reduction in grey and white matter concentration and increases in CSF concentration, based on the probability of a voxel belonging to one of the three tissue classes.

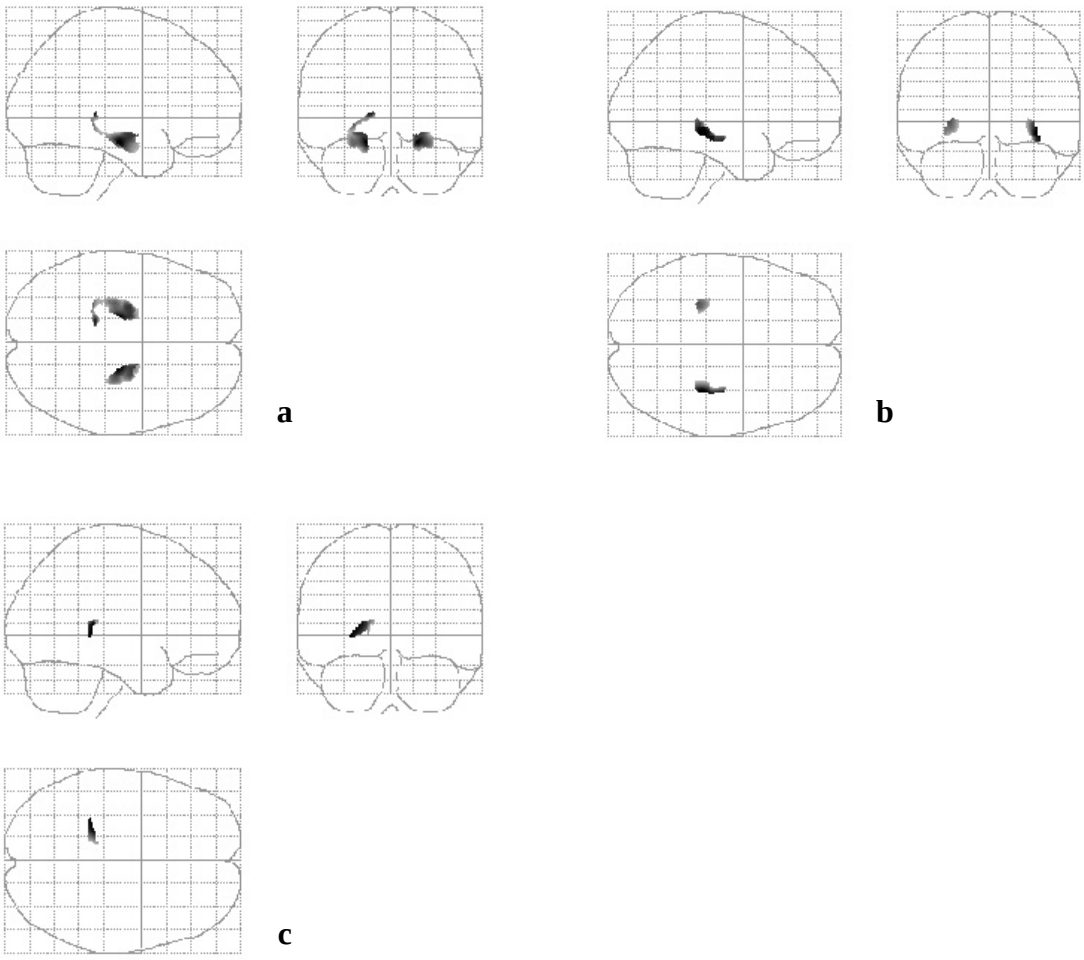


Figure 7.8. Grey matter (a) and white matter decreases (b) and CSF increases (c) between patient group at 1 month and controls

Bilateral decreases in grey matter concentration were found in the parahippocampal gyri. White matter concentration also decreased bilaterally in left and right sub-gyri. These findings were accompanied by an increase in CSF concentration in the left hippocampal region with the peak coordinate positioned in left sub-gyri. No increase in CSF concentration was found in the right hippocampal region. However, cluster sizes indicate that in comparison to the control group, the patient group had a greater decrease in accumulated grey and white matter concentration in the left hippocampus than the right supporting the current finding of increased CSF concentration in the left hippocampal region.

Table 7.8. Hippocampal Differences Between Patient Group at 1-month and Controls (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
Increases					
343	L Sub-gyral CSF	-30	-38	-1	3.07*
Decreases					
2110	L Parahippocampal gyral GM	-18	-17	-18	3.93**
1241	R Parahippocampal gyral GM	20	-18	-17	3.85**
660	R Extra-nuclear WM	35	-21	-8	3.35**
325	L Sub-gyral WM	-31	-33	0	2.63*

GM – Grey Matter; WM – White Matter
*Significant at $p<0.01$ height threshold, **Significant at $p<0.001$ height threshold

Peak voxel coordinates for left sub-gyral CSF and left sub-gyral white matter were significant at the higher height threshold of $p < 0.01$ whereas peak coordinates for left and right parahippocampal gyral GM and right extra-nuclear white matter were significant at height thresholds of $p < 0.01$ and $p < 0.001$.

Hippocampal Differences between Patient Group at 6-Months and Controls

At 6 months post injury, a decrease in grey and white matter concentration between the patient group and control group was found with significant grey matter clusters located bilaterally in parahippocampal gyri and extra-nuclear white matter in the left limbic lobe. A bilateral increase in lateral ventricular CSF concentration was also present with the left lateral ventricle showing a larger decrease than the right. This supports the current findings of decreased grey and white matter concentration where grey matter reduction was greater in the left parahippocampal gyrus than the right and extra-nuclear white matter concentration was limited to the left hippocampal region.

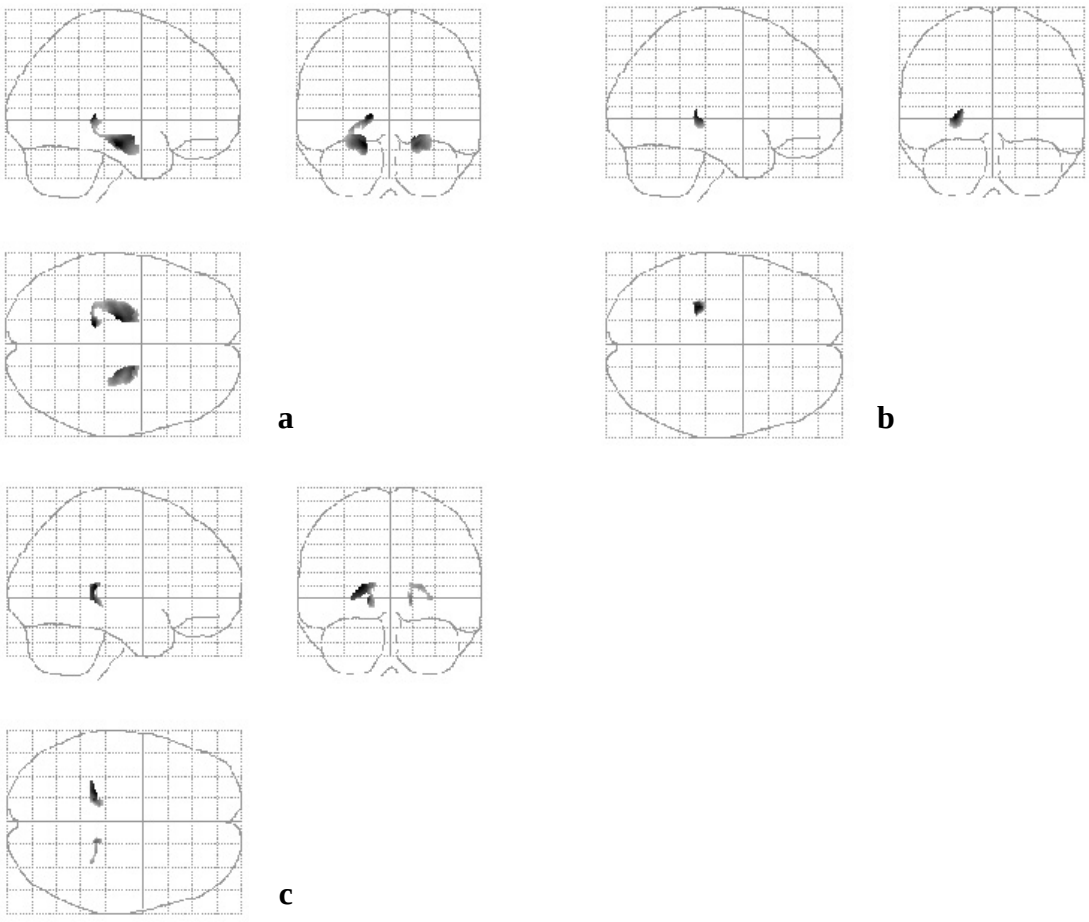


Figure 7.9. Grey matter (a) and white matter decreases (b) and CSF increases (c) between patient group at 6 months and controls

Table 7.9. Hippocampal Differences Between Patients at 6 Months and Controls (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
Increases					
495	L Lateral ventricular CSF	-23	-35	8	2.99*
206	R Lateral ventricular CSF	24	-38	9	2.34
Decreases					
2304	L Parahippocampus gyral GM	-15	-36	0	4.44**
1399	R Parahippocampus gyral GM	20	-18	-18	3.70**
367	L Extra-nuclear WM	-27	-33	2	2.59

GM – Grey Matter; WM – White Matter
*Significant at $p<0.01$ height threshold, **Significant at $p<0.001$ height threshold

Of the 5 clusters tabulated in table 7.9, the peak voxel coordinate for the left CSF cluster was found to be significant at a higher height threshold of $p < 0.01$. Both grey matter clusters in the left and right parahippocampal gyri had peak voxels which were significant at $p < 0.01$ and $p < 0.001$.

Analysis was also carried out to determine changes in hippocampal tissue concentration between patients at 1 and 6-months post injury. However, no significant results were found at thresholds $p < 0.001$, $p < 0.01$ or $p < 0.05$.

Analyses of Hippocampal Grey Matter Concentration and Neuropsychology

The effect of head injury on the hippocampal region and neuropsychological consequences was studied. The analysis used VBM to investigate correlations of neuropsychological test scores with images specifically masked for the hippocampus. With previous VBM analyses, more extensive differences at both 1 and 6 months were found with grey matter segments. Subsequently, the current analysis of tissue class and neuropsychological data focussed on grey matter which is consistent with the majority of hippocampal studies using VBM due to the hippocampus predominantly consisting of grey matter. Exploratory analyses of data using white matter and CSF segments did not provide consistent interpretable results when analysed with neuropsychological data probably due to limitations of segmentation into different tissue classes. Hence, the results of grey matter analyses are reported. Significance was assessed using uncorrected thresholds of $p < 0.001$, $p < 0.01$ and $p < 0.05$. Where analyses had no *a priori* information, significance is reported at an uncorrected height threshold of $p<0.05$, corrected for multiple comparisons at the

cluster level ($p < 0.05$). For analyses where there were *a priori* hypotheses, significance was assessed at an uncorrected height threshold of $p < 0.05$ and clusters were not corrected for multiple comparisons (Friston 1997; Keller et al., 2002). Significant clusters were then analysed to determine if they survived correction for multiple comparisons and reported as such for consistency alongside peak voxel coordinates.

Hippocampal Correlations with the NART Error Score

Correlational analysis with the NART error score was performed to identify if it had to be included as a confounding covariate. Analyses using white matter segmented images showed no results; negative grey matter and CSF correlation revealed 5 and 3 non-significant clusters respectively. Despite this non-significance in relation to hippocampal morphology, SPSS analyses in the current study found correlations with the NART error score and 1-month post injury neuropsychology scores (Digit Span and total BVRT errors) and also 6-months post injury scores (Immediate and Delayed Recall, Digit Span, SDMT Verbal and COWAT). Therefore, it was necessary to include the NART error score as a confounding covariate in the design matrix to control for possible erroneous results and to maximise consistency throughout statistical analysis of images.

Correlations of 1-Month Post injury Grey Matter Images with Test Scores

The segmented and masked MR images at 1 month post injury were correlated with 1 month post injury neuropsychological data. One patient had completed

neuropsychological assessment at 1 month but had failed to complete assessment at 6 months. Therefore, this patient was omitted from analyses of neuropsychological test data. For all analyses at 1 month post injury, n = 46 except Grooved Pegboard where n = 44 for non-dominant hand scores and n = 45 for dominant hand scores.

Table 7.10. GMC Clusters Resulting from Correlations of 1 Month Post Injury Segmented Images and Neuropsychological Test Scores at 1 Month Post injury (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	z	y	Peak Z Score
Delayed Recall					
550	L Parahippocampal gyral GM	-28	-7	-15	2.95
Digit Span					
439	R Uncus GM	21	-5	-25	2.71
Pegboard: Non-Dominant Hand					
760	L Hippocampal GM	-31	-32	-8	3.43*

GM – Grey Matter; GMC – Grey Matter Concentration; *Significant at $p < 0.001$ height threshold

Delayed Story Recall

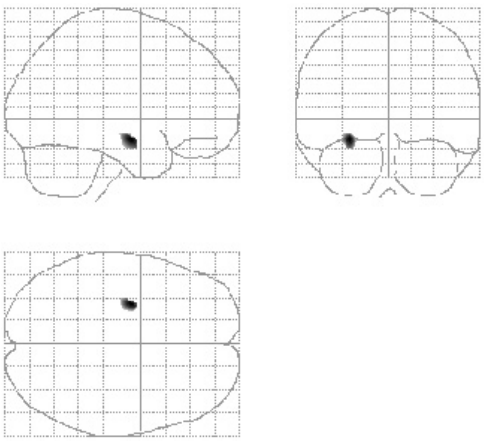


Figure 7.10. Positive grey matter correlation with Delayed Story Recall score

Positive correlation was found with delayed story recall score and grey matter concentration in the left parahippocampal gyrus with a cluster of 550 voxels. As story recall scores consisted of the total number of correct answers, the VBM correlational analysis suggests that lower scores are associated with decreased grey matter concentration in the left hippocampal region due to brain atrophy as a result of head injury. Immediate story recall at 1 month was also analysed with images at 1 month but no clusters were significant at the uncorrected height threshold of $p < 0.05$ or survived multiple correction at the cluster level.

Digit Span

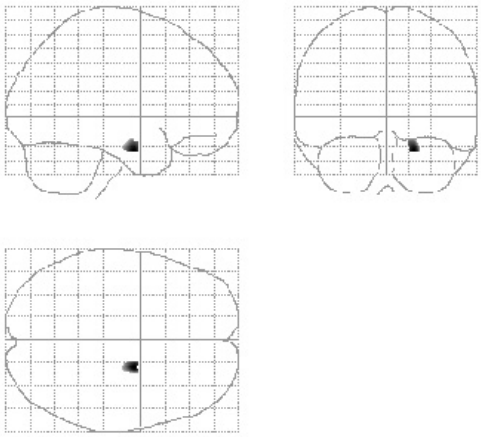


Figure 7.11. Positive grey matter correlation with Digit Span score

Grey matter concentration was found to correlate positively with digit span score with a cluster containing 439 voxels in the right uncus, the most anterior part of the parahippocampal gyrus. Digit span score is a measure of the number of digits correctly recalled and is a composite of both forward and backward digit recall. Therefore, these results suggest that decreased grey matter concentration in the hippocampal region is associated with lower digit span score.

Grooved Pegboard using the Non-Dominant Hand

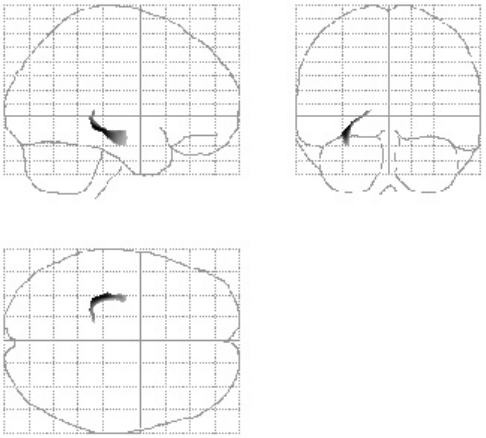


Figure 7.12. Negative grey matter correlation with Grooved Pegboard scores using the non-dominant hand

Grey matter concentration was found to be negatively correlated with a cluster located in the left hippocampus and the peak voxel was also significant at height thresholds of $p<0.01$ and $p<0.001$. This result suggests that a decrease in grey matter concentration is associated with longer completion times for the Grooved Pegboard task using the non-dominant hand.

Correlations of 6-Month Post injury Grey Matter Images with Test Scores

The hippocampal masked MR images at 6-months post injury were correlated with 6-month post injury neuropsychological data. As with analysis of 1-month test scores, 1 patient was omitted due to incomplete test scores at 6 months providing a patient group where n = 46 in all but Simple and Choice Reaction Time tests where n = 40 due to an equipment malfunction. All results were recorded at a height threshold of $p < 0.05$ and corrected for multiple comparisons.

Table 7.11. GMC Clusters Resulting from Correlations of 6 Months Post Injury Segmented Images and Neuropsychological Test Scores at 6 Months Post Injury (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
Immediate Story Recall					
491	R Hippocampal GM	27	-38	-2	4.89**
Digit Span					
851	R Hippocampal GM	28	-9	-20	2.50
Benton Visual Retention Test					
1084	R Parahippocampal gyral GM	19	-11	-18	2.92*
385	L Parahippocampal gyral GM	-17	-13	-16	1.97
Pegboard: Non-Dominant Hand					
797	L Sub-gyral GM	-31	-31	-11	4.53**
Controlled Oral Word Association Test					
884	L Hippocampal GM	-27	-14	-20	2.43
456	R Hippocampal GM	26	-11	-20	1.91
Rey Figure Test: Delayed Recall					
472	R Hippocampal GM	33	-22	-13	2.32
SDMT: Verbal					
650	L Parahippocampal gyral GM	-30	-16	-14	2.42
1230	R Parahippocampal gyral GM	26	-12	-22	2.45
SDMT: Written					
510	L Hippocampal GM	-30	-16	-14	2.36
Trail Making: A					
1767	R Parahippocampal gyral GM	21	-16	-20	2.8*
Trail Making: B					
1209	R Hippocampal GM	26	-14	-21	2.44*
Choice Reaction Time: Decision Time					
1207	R Parahippocampal gyral GM	21	-7	-17	2.57
Simple Reaction Time: Decision Time					
1029	R Parahippocampal gyral GM	16	-6	-13	2.60

GMC – Grey Matter Concentration; GM – Grey Matter

*Significant at $p < 0.01$ height threshold, **Significant at $p < 0.001$ height threshold

Immediate Story Recall

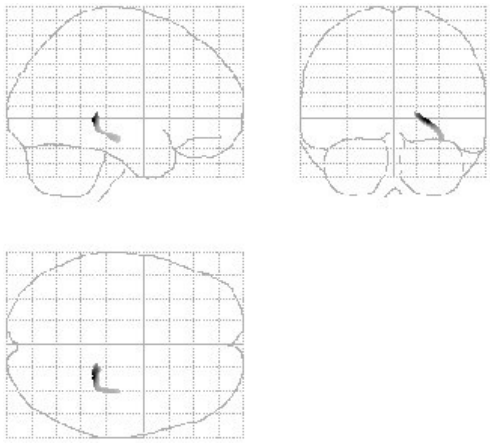


Figure 7.13. Positive grey matter correlation with Immediate Story Recall score

Grey matter concentration was found to be positively correlated with immediate story recall score represented by a cluster in the right hippocampus. The peak voxel of this cluster was significant at higher height thresholds of $p < 0.01$ and $p < 0.001$. Since story recall is based on the number of correct answers, this correlational analysis suggests that poorer recall is associated with decreased grey matter concentration.

Digit Span

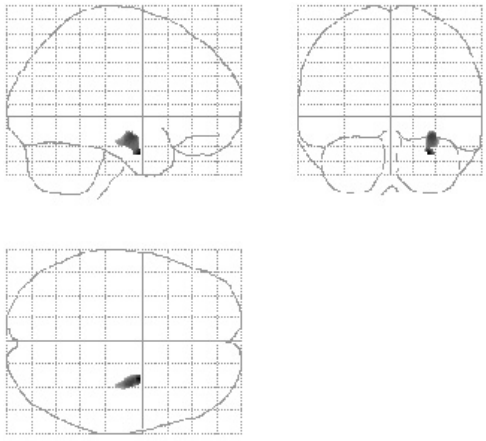


Figure 7.14. Negative grey matter correlation with Digit Span score

Digit span score was found to be negatively associated with grey matter concentration represented by a right hippocampal grey matter cluster of 851 voxels. This result seems to suggest that higher digit span score is associated with decreased concentration of grey matter.

Benton Visual Retention Test

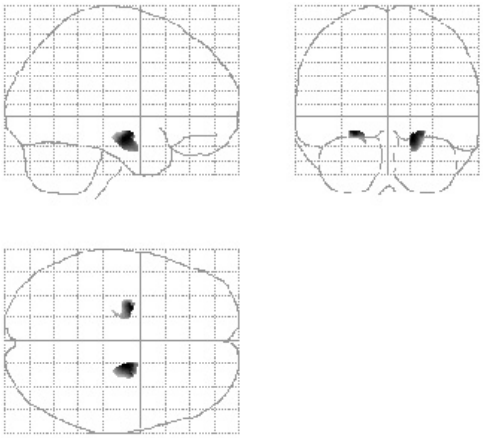


Figure 7.15. Negative grey matter correlation with BVRT score

No association with BVRT error scores and tissue concentration was established at 1 month. However at 6 months, negative association was found with grey matter concentration with a large grey matter cluster of 1084 voxels located in the right parahippocampal gyrus and a smaller cluster of 385 voxels located in the left parahippocampal gyrus. The larger cluster had a peak voxel which was also significant at $p < 0.01$. The scoring of the BVRT was taken as a measure of the number of errors made during the task. Thus, the correlational results indicate that an increase in errors is associated with a decrease in grey matter concentration.

Grooved Pegboard using the Non-Dominant Hand

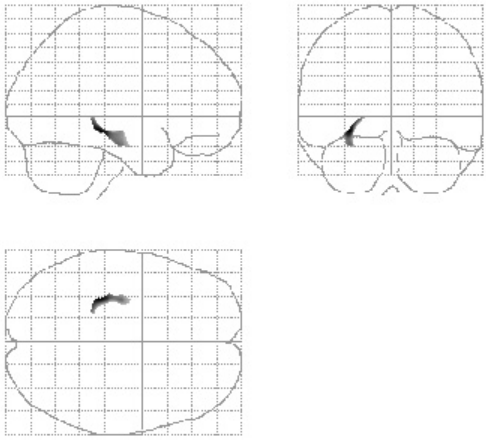


Figure 7.16. Negative grey matter correlation with Grooved Pegboard using the non-dominant hand

Grey matter concentration was found to be negatively associated with Grooved Pegboard scores using the non-dominant hand with a cluster of 797 voxels in the left sub-gyrus. The peak voxel of this cluster was also significant at height thresholds $p < 0.01$ and $p < 0.001$. These results are similar to the results at 1 month where grey matter concentration was also negatively associated with grooved pegboard scores using the non-dominant hand. Thus, suggesting that time taken for task completion is increased with regard to a decrease in grey matter concentration.

Controlled Oral Word Association Test

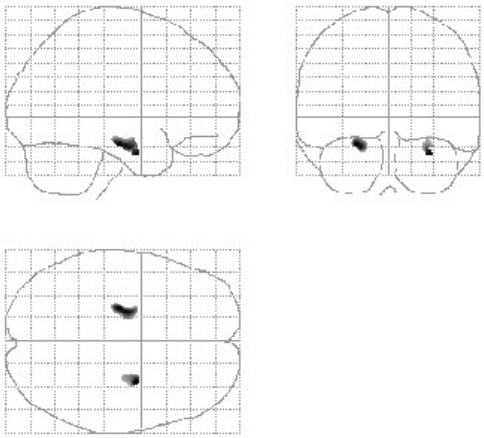


Figure 7.17. Positive grey matter correlation with COWAT scores

Grey matter concentration was found to be positively associated with COWAT score. Bilateral clusters of grey matter concentration were located in the left and right hippocampi with clusters consisting of 884 and 456 voxels respectively. COWAT scores are calculated based on the total of appropriate words in the word-naming trials which, when correlated with tissue class, suggests that lower scores are attributed to a decrease in grey matter concentration.

Rey Figure Test: Delayed Recall

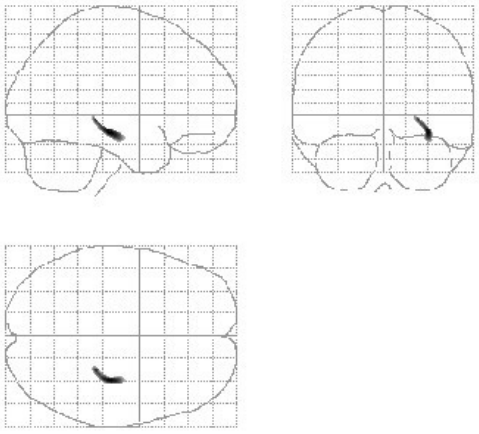


Figure 7.18. Positive grey matter correlation with Rey Figure scores

The Rey Figure delayed recall scores were positively associated with grey matter concentration in the right hippocampus with a cluster of 462 voxels. The Rey Figure scores were calculated to give the total number of correctly placed units within the design. Therefore, the results of the correlations imply that lower scores are associated with decreased grey matter concentration.

Symbol Digit Modalities Test – Verbal

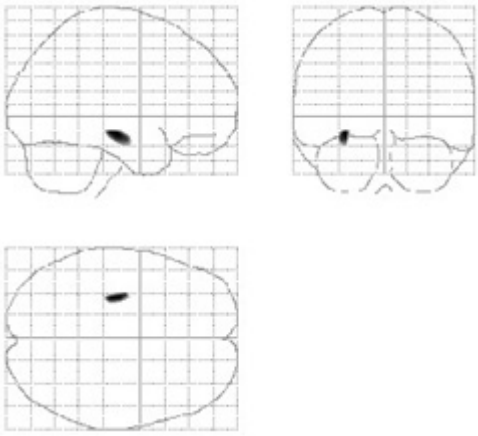


Figure 7.19. Positive grey matter correlation with SDMT Verbal scores

The score for the verbal component of the Symbol Digit Modalities Test consisted of the number of correct responses and was positively correlated with grey matter concentration with a cluster of 650 voxels in the parahippocampal gyrus. This indicates that a decrease in grey matter concentration is associated with lower scoring in the verbal component of the SDMT.

Symbol Digit Modalities Test – Written

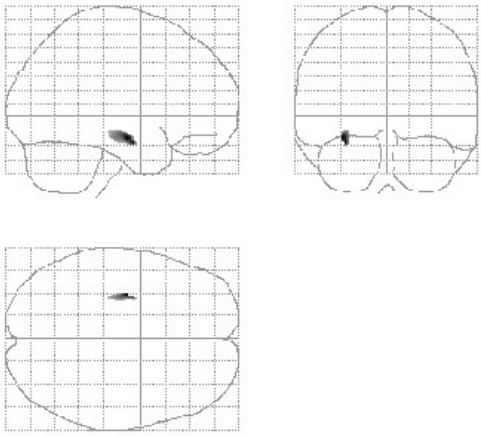


Figure 7.20. Positive grey matter correlation with SDMT Written scores

A cluster of grey matter concentration containing 510 voxels was located in the left hippocampus. The results of the correlation with the written component of the Symbol Digit Modalities Test and tissue class mirrored those of the verbal component whereby grey matter concentration was found to be positively associated suggesting that an increase in grey matter concentration is associated with higher scores.

Trail Making – A

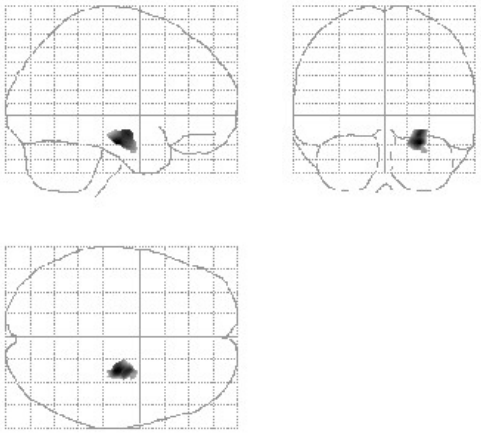


Figure 7.21. Positive grey matter correlation with TMT A scores

Grey matter concentration was found to be positively correlated with the first of the Trail Making Tests (A). A cluster consisting of 1747 voxels was found in the right parahippocampal gyrus and the peak voxel was found to be significant at the higher height threshold of $p < 0.01$. Due to the TMT score being measured in seconds with better performance indicated by the least amount of time needed to complete the task, these results appear to be counterintuitive as they suggest that a decrease in grey matter concentration is associated with decreased time taken to complete the task.

Trail Making – B

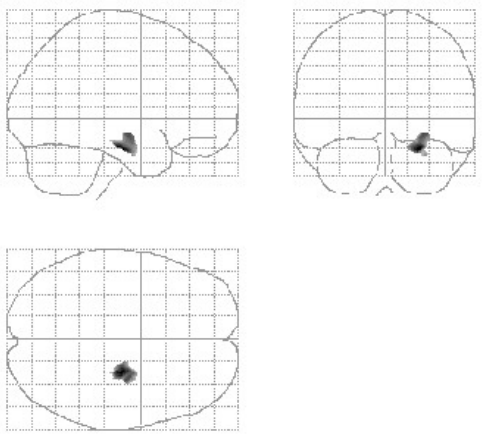


Figure 7.22. Positive grey matter correlation with TMT B scores

With the second Trail Making Test (B), the result mirrored that of the first Trail Making Test (A) whereby grey matter concentration was found to be positively associated. A cluster of 1209 voxels was located in the right hippocampus the peak voxel of which, was also significant at height threshold $p < 0.01$.

Choice Reaction Time – Decision Time

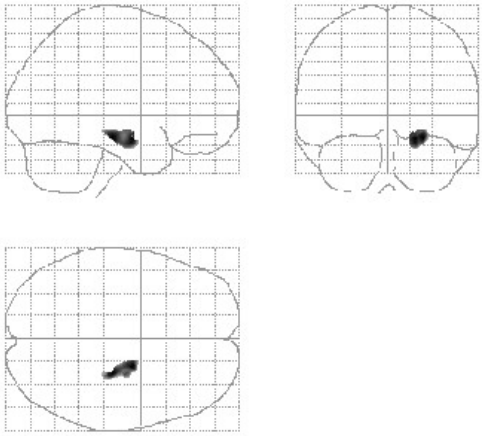


Figure 7.23. Negative grey matter correlation with MCRT Decision scores

For the decision time of the Choice Reaction Time test, grey matter concentration was negatively correlated with test completion time with a grey matter cluster of 1207 voxels located in the right parahippocampal gyrus. Reaction time was measured in seconds where improved performance was indicated by the least amount of time taken to complete each part of the task. Negative correlation indicates that increased reaction time is associated with a decrease in concentration of grey matter.

Simple Reaction Time – Decision Time

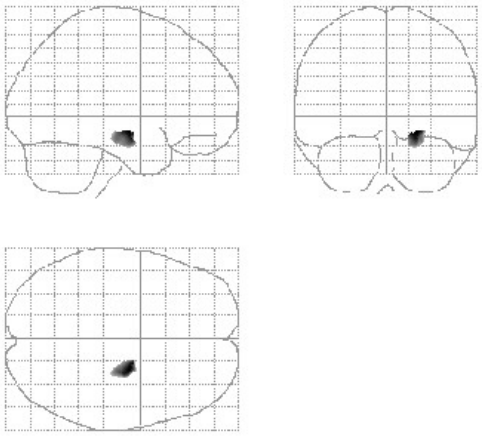


Figure 7.24. Negative grey matter correlation with MSRT Decision scores

The decision time for the Simple Reaction Time test was negatively associated with grey matter with a grey matter concentration cluster of 1029 voxels being located in the right parahippocampal gyrus. These results for the decision time of the MSRT test mirror those of the decision time of the MCRT test where grey matter concentration was also negatively correlated with a cluster located in the right parahippocampal gyrus. The results imply that a reduction in grey matter concentration in the right hippocampal region is associated with an increase in decision reaction time.

Summary of VBM and Neuropsychological Data

Although all neuropsychological test scores were analysed, for 1 month scores only results for delayed recall, digit span and Grooved Pegboard using the non-dominant hand were significant whereas all 6 month test scores showed significant correlations except for delayed story recall, Grooved Pegboard with the dominant hand and Simple and Choice movement times. All results were thresholded at $p < 0.05$ and corrected for multiple comparisons at the cluster level.

For 1-month scores, all correlations with test performance were in the expected direction with regard to increased or decreased grey matter concentration; that is, that decreases in grey matter concentration were associated with poorer test performance. For 6-month scores, correlation with grey matter concentration resulted in 11 of the 14 grey matter clusters in the expected direction with regard to test performance (table 7.12).

Table 7.12. Number of Significant Clusters Found in Correlational Analyses Between Grey Matter Concentration and Neuropsychological Tests

Task	No. of Clusters with Positive Correlations	No. of Clusters with Negative Correlations
1 Month		
Delayed Story Recall	**1**	0
Digit Span	**1**	0
Pegboard: Non-Dominant Hand	0	**1**
Total 1-Month Clusters:	2	1
6 Months		
Immediate Story Recall	**1**	0
Digit Span	0	1
Benton Visual Retention Test	**2**	0
Pegboard: Non-Dominant Hand	0	**1**
Controlled Oral Word Association	**2**	0
Rey Figure Test	**1**	0
Symbol Digit Test: Verbal	**1**	0
Symbol Digit Test: Written	**1**	0
Trail Making: A	1	0
Trail Making: B	1	0
Choice Reaction Time: Decision	0	**1**
Simple Reaction Time: Decision	0	**1**
Total 6-Month Clusters:	10	4

Numbers in bold indicate number of correlations in the expected direction.

Correlations of Grey Matter with Glasgow Coma Scale

Hippocampal grey matter segments were investigated with regard to clinical variables due to the vulnerability of the hippocampus in brain injury. Hippocampal atrophy has been documented following brain injury (Verger et al., 2001) and has been associated with injury severity and memory outcome (Tate & Bigler, 2000). Segmented images at 1 and 6 months post injury were correlated with Glasgow Coma Scale at A&E (GCS at A&E).

Table 7.13. Positive Correlations of GCS at A&E with Grey Matter Images (Height Threshold = $p<0.05$; Clusters Corrected for Multiple Comparisons, $p<0.05$)

Cluster Size (k)	Talairach Region	MNI Coordinates (mm) x	y	z	Peak Z Score
GCS at A&E: 1 month					
702	L Hippocampal GM	-31	-15	-14	2.91
GCS at A&E: 6 months					
1041	L Hippocampal GM	-33	-22	-12	4.12*

GM – Grey Matter; *Significant at $p<0.001$ height threshold

GCS at A&E with Images at 1 month

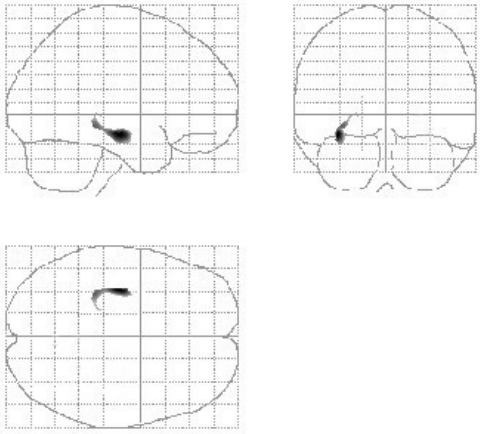


Figure 7.25. Positive grey matter correlation with GCS at A&E scores at 1 month post injury

Grey matter concentration at 1 month was positively associated with GCS at A&E score with a cluster of 702 voxels in the left hippocampus. With regards to injury severity, a higher GCS score accompanies a more favourable level of consciousness. Correlation with grey matter concentration implies that increased concentration is associated with higher GCS score.

GCS at A&E with Images at 6 months

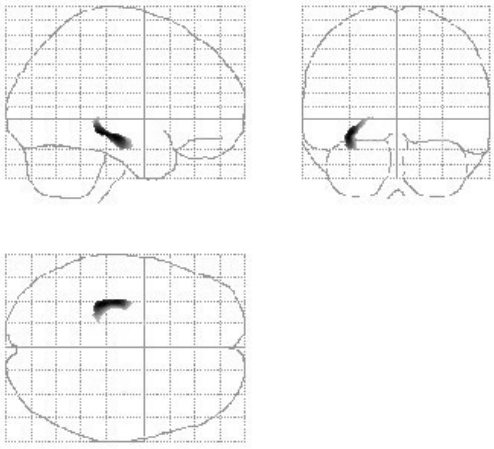


Figure 7.26. Positive grey matter correlation with GCS at A&E scores at 6 months post injury

As with images at 1 month, analysing images at 6 months with GCS at A&E score produced positive association with grey matter concentration. A grey matter cluster consisting of 1041 voxels was found in the left hippocampus and had a peak voxel which was significant at height thresholds $p < 0.01$ and $p < 0.001$. As with the GCS scores at 1 month, the results suggest that higher GCS scores at A&E are associated with increased grey matter concentration.

Discussion

Wholescan Signal Change and Age

Various studies have looked at the effects of age in the human brain (e.g. Good et al, 2001). The current study showed age to be negatively associated with signal in wholescan VBM of the patient group. Clusters were located in the left pyramis of the cerebellum, the corpus callosum and in the right temporal lobe. Previous research has found modest age effects in the corpus callosum (Driesen & Raz, 1995;

Johnson et al., 1994; Pozzilli et al., 1994) and parallel region-of-interest measurements in the current study also found modest callosal age effects. Age effects in the cerebellum have been found in a group of 190 healthy volunteers aged 18-81 (Raz et al., 2001) and in a group of 48 healthy volunteers aged 20-71 (Luft et al., 1999) supporting the current findings of age effects in this region. There is also support for the finding of age effects in the temporal lobe. Previous research has found age-related reduction in the medial temporal lobes of healthy adults aged 26-82 years (Raz et al., 2004) and age-related effects in temporal grey and white matter in 87 healthy subjects aged 22-88 years (Allen et al., 2005).

Whole Scan Signal Change between Patients and Controls

Decreases in signal were detected between the patient group at 1 and 6 months post injury. A single large cluster was detected largely in the right frontal lobe which encompassed orbital, superior, inferior and medial regions and extended inferiorly into the left frontal lobe. As with the temporal lobes, the frontal lobes are vulnerable in head injury due to their position in the brain; they are covered by uneven bony surfaces which, due to forces exerted during injury, can cause damage regardless of the site of impact. Damage to the frontal lobes often causes deficits in executive functioning, learning and memory (Lehtonen et al., 2005; Wilson et al., 1995; Mattson & Levin, 1990) and these deficits have been found in patients with either focal or diffuse injury (Wallesch et al., 2001; Spikman, Deelman & Van Zomeren, 2000). Such findings in diffuse injury imply that this type of injury alone is sufficient to cause frontal lobe dysfunction similar to that found with actual lesioned

frontal tissue. Therefore, a patient without lesions as detected by MR imaging does not necessarily rule out damage to the frontal lobe.

Signal decrease was also evident in the left frontal lobe when the patient group was compared to controls. At 6 months post injury, the cluster in the left frontal lobe was only significant at a lower threshold suggesting that damage in this region had subsided over time and residual damage was only identified by VBM at this lower threshold. At 1 month post injury, signal decrease was also found in the anterior cingulate. This structure is located on the medial surface of the frontal lobes and has connections with cortical and subcortical brain structures including basal ganglia, association cortex and motor and limbic systems. It is involved in executive functions and has been shown to be activated in relation to increased task difficulty (Barch et al., 1997). This has been demonstrated in a PET study by Levine and colleagues (2002) where moderate to severely head injured patients with focal and diffuse injury were imaged 4 years post injury; frontal lobe and anterior cingulate activation was greater than in control subjects when completing a memory retrieval task (Levine et al., 2002). PET analysis has also demonstrated hypometabolism in the prefrontal cortex and anterior cingulate in severely head-injured patients where decreased cortical metabolism related to cognitive and behavioural disorders (Fontaine et al., 1999).

To date, there has not been a vast amount of research using VBM to investigate head injury. Despite this, the research which has been done has corroborated results of research using neuropsychological investigation and other imaging techniques. SPM analysis using SPECT on head injured groups containing focal and diffuse injuries

found frontal lobe and anterior cingulate abnormalities when compared to a patient group (Stamatakis et al., 2002). VBM has detected reduced grey matter density in the basal forebrain, hippocampal formation and neocortical regions as well as reduced grey matter concentration in frontal and temporal cortices, cingulate gyrus, subcortical grey matter and the cerebellum in cohorts of head injured patients compared to controls (Salmond et al., 2005; Gale et al., 2005).

To the author's knowledge, there has been no VBM analysis reported investigating head injury using whole scan VBM methodology. Previous whole scan research has focused on SPECT and lesional analysis of patients whose brain injury was caused by various aetiologies such as semantic dementia, stroke and herpes encephalitis (Stamatakis & Tyler, 2005; Tyler, Marslen-Wilson & Stamatakis, 2005b). The current findings of reduced signal in the frontal lobes and anterior cingulate of head injured patients is supported by previous research and suggests that, when used alongside analysis of neuropsychological scores, it may provide further understanding of the neural circuitry involved in cognitive processes; the neural circuitry of the frontal lobe and anterior cingulate may underlie several of the cognitive deficits commonly reported after head injury.

Cause of Injury as Analysed by Whole Scan VBM Analysis

Global signal change in relation to cause of injury was determined by separating the patient group into smaller groups dependent on cause of injury – assault and falls. When compared with controls, analysis of 1 month scans in the assault group revealed signal decreases in the tuber vermis of the cerebellum and superior frontal

gyrus. Damage to the cerebellum in this group may be the result of the head being struck from behind which is a common occurrence in assault cases. This could also explain the damage to the frontal lobe which would have been caused by contrecoup injury. Analysis of 6 month scans in the assault group revealed signal change in the superior frontal gyrus with the same peak voxel as that of the frontal cluster at 1 month. The cluster at 6 months was substantially reduced in size and there was no signal decrease in the cerebellum indicating alleviation of injury over time.

Results in the fall group presented signal decrease in the cingulate gyrus at 1 month post injury and signal decrease in the cingulate gyrus accompanied with the left lateral ventricle at 6 months. The signal decrease evident in the cingulate gyrus is further evidence that this structure is vulnerable in head injury and may contribute to cognitive sequelae of head injury. Further to this, the cluster size at 6 months was larger than at 1 month post injury suggesting progressive neuronal damage in this structure over the 5-month period. This larger cluster at 6 months was accompanied with signal decrease in the lateral ventricle further strengthening the argument of progressive neuronal damage as ventricular enlargement in head injured patients has been previously described (Ariza et al., 2004; Henry-Feugeas et al., 2000; Blatter et al., 1997).

Hippocampus and Age

Previous research using VBM has documented that the hippocampus and other limbic structures have relative grey matter preservation (Grieve et al., 2005; Good et al., 2001) but hippocampal age effects have been found to influence autobiographical

memory (Maguire & Frith, 2003) and grey matter volume decline related to aging has been found in men but not women (Pruessner et al., 2001). Studies using other methodology have also produced inconsistent results where no correlations of age with hippocampus have been found (Sullivan, Marsh & Pfefferbaum, 2005; Bigler et al., 1997) and where correlations have been found (Driscoll et al., 2003; Juottonen et al., 1999).

Imaging studies have shown that aging in the brain is associated with increased CSF and decreased grey and white matter (Ge et al., 2002; Courchesne et al., 2000; Jernigan, Press & Hesselink, 1990). This gives support to the current findings where age was associated with decreased grey and white matter concentration and increased CSF concentration at both 1 and 6 months post injury. However, it should be noted that white matter concentration at both time intervals was associated with age in the left hippocampal region rather than bilaterally which would normally be expected and was the case for grey matter and CSF concentration. Although unreliable, the finding of increased CSF concentration is probably reflective of increased ventricular size associated with increased age.

Hippocampal Atrophy as a Result of Head Injury

Atrophy in the hippocampal region in the form of decreased grey and white matter concentration was found in the head injured group at both time intervals when compared to controls and these deficits were accompanied by increased CSF concentration. At both 1 and 6 months, there was greater reduction in grey than white matter concentration with images at 6 months producing a unilateral white

matter cluster suggesting that VBM is more robust with grey matter segmented images. Decreases and increases in grey matter and CSF concentration respectively were greater at 6 months which is consistent with the knowledge that the pathological sequelae that results from head injury are time-dependent. Although the increase in CSF in the hippocampal region was small, this too is consistent with the current literature which reports that the greatest change in CSF in head injured patients is seen in the ventricles (Serra-Grabulosa et al., 2005; Blatter et al., 1997).

As with the region-of-interest findings reported in chapter 5, comparison of the hippocampal region between the patient group at 1 and 6 months post injury yielded no results. This suggests that changes within the patient group which may have resulted due to injury were not substantial enough between 1 and 5 months to be detected by VBM. This result mirrors the region-of-interest findings which detected no hippocampal volume change in the patient group between 1 and 5 months post injury. Reduced hippocampal volumes have been demonstrated to be greater in head injured patients imaged more than 100 days after injury than patients imaged up to and including 100 days after injury when compared to controls (Bigler et al., 1997). The aforementioned patient group had a mean GCS of 7.97 and 7.12 indicating a severely head injured population whereas the current study had a mean GCS of 13.1 indicating mild head injury. As discussed previously, the severity of injury of different subject groups may go some way to explaining the lack of atrophy as a function of time due to the relationship of injury severity and degree of atrophy (Tate & Bigler, 2000).

Hippocampal Region and Memory

To date, there has only been one paper published describing the results of using VBM to investigate cognitive deficits in head injured patients (Gale et al., 2005). Therefore, the current findings are presented in relation to studies using other methodology unless stated otherwise. Table 7.12 indicates that for the current study, results from grey matter are more robust and reliable and as such, discussion of neuropsychological correlational results is confined to grey matter. VBM analysis was used to determine association of memory with hippocampal region tissue concentration. Memory was investigated using immediate and delayed story recall, the Benton Visual Retention Test (BVRT) and delayed recall of the Rey Figure. The hippocampus has been shown to be involved in memory and for the aforementioned memory tests used in the current study, correlations in the expected direction with regard to head injury were found. For delayed story recall at 1 month the left hippocampal region showed associations with grey matter concentration and immediate recall at 6 months showed associations with grey matter in the right hippocampal region. The finding that damage in the left hippocampal region at 1 month was not present at 6 months may be explained by alleviation of injury at this site over time. Also, pathological damage to the right hippocampal area may not have been substantial at 1 month with regard story recall, as to be detected by VBM.

Although evidence suggests that the left hippocampus is involved with verbal memory (Frisk & Milner, 1990) and the right hippocampus is involved in non-verbal memory such as visual and spatial memory (Smith & Milner, 1981), it is still a contentious issue. In head injured populations, correlations of verbal memory have been found with both left and right hippocampal volumes (Bigler et al., 1997) while

others have found no hippocampal association with story recall (Tomaiuolo et al., 2004) or have reported atrophic changes in the left but not the right hippocampus (Bigler et al., 1996). The current VBM findings add to the view that strict lateralisation of hippocampal memory functions may not be the case.

Visuo-spatial memory was investigated in the current study by using delayed recall of the Rey Figure and the Benton Visual Retention Test (BVRT). No associations were found with BVRT at 1 month. At 6 months, associations were found with grey matter and BVRT and the Rey Figure; bilateral decreased grey matter concentration was associated with poorer memory performance suggesting atrophy in these hippocampal regions. It is of interest to note that reduction in grey matter concentration was associated with the right hippocampal region for both BVRT and the Rey Figure. This may be weak evidence for lateralisation of non-verbal memory such as visuo-spatial memory in the right hippocampal region. Non-verbal memory has been associated with the right hippocampus in TBI patients (Tomaiuolo et al., 2004) and greater spatial recall after left temporal resection (Pillon et al., 1999). However, other research has shown poor visual recall in patients with both left and right MTS when compared with controls (Miller, Munoz & Finmore, 1993), no association of the right hippocampus with visuo-spatial recall (Kilpatrick et al., 1997) and no association of visual memory with either hippocampus (Bigler et al., 1997). Although the current results regarding the right hippocampal region may lean towards lateralisation of non-verbal memory, it must be noted that with BVRT, reduced grey matter concentration was also associated with the left hippocampus confounding the theory of lateralisation of function.

Correlations of digit span with grey matter concentration were found in the right hippocampal region at both 1 and 6 months post injury. The association at 1 month suggests poorer digit recall with decreased grey matter concentration but the association at 6 months counterintuitively implies the opposite, that poorer performance of digit span was associated with increased grey matter concentration. As digit span measures attention and short-term memory which are thought to be frontal lobe functions, correlations with grey matter concentration in the hippocampal region were speculative and any findings could be due to this region having been affected by general diffuse injury. Digit span has been shown to be insensitive to the effects of head injury (Brooks, 1975), which may account for spurious results. Also, as this was an exploratory analysis, acceptance of the null hypothesis leading to type II errors cannot be ruled out.

Hippocampal Region and other Neuropsychological Test Scores

Visual-motor integration and mental processing speed were assessed using the Grooved Pegboard, Symbol Digit Modalities and Trail Making tests. Associations of atrophy in the hippocampal region were found with Grooved Pegboard using both the dominant and non-dominant hand at 1 month and with the non-dominant hand at 6 months. Thus, suggesting that decreased grey matter concentration at 1 and 6 months were associated with poorer performance.

Both verbal and written components of the Symbol Digit Modalities Test (SDMT) showed association with reduced grey matter concentration indicating poorer test

performance. This is consistent with the hypothesis that the hippocampal region is susceptible to diffuse damage after head injury.

With analysis of the Trail Making Tests A and B, there were associations with grey matter concentration but in the wrong direction; that is, the findings suggest that poorer performance was associated with increased grey matter concentration. The raw scores were checked for outliers and extreme values but nothing untoward was discovered. Therefore, it is likely that these irregular results are due to a segmentation issue.

Reaction Time tests were used to detect changes in processing speed that may have been compromised as a result of diffuse damage. Impaired reaction time has been documented previously in severely head injured patients (Sarno et al., 2003; Nissley & Schmitter-Edgecome, 2002) and also in very mild (GCS = 15) head injured patients (Voller et al., 1999). Salmond and colleagues (2005) performed a VBM analysis of the basal forebrain, hippocampal formation and neocortical regions and reported a diffuse pattern of injury with reduced grey matter in these regions when compared to controls. Neuropsychological testing of this patient group found slower reaction time in comparison to a control group (Salmond et al., 2005). The current findings provide evidence for susceptibility of the hippocampal region in head injury as slower reaction time was found to be associated with decreased grey matter concentration.

Verbal fluency was measured using the Controlled Oral Word Association Test (COWAT). Previous research has found reduced COWAT performance in head

injured patients when compared to controls in moderate and severe head injury (Mathias et al., 2004; Schmitter-Edgecombe & Woo, 2004) and a trend has been observed in very mild head injury (GCS = 15) but was not significant (Voller et al., 1999). Also, COWAT score has shown to be unaffected following mild head injury in patients assessed 4 weeks post injury (Mathias, Beall & Bigler, 2004). In the current study, lower scores on the COWAT were associated with bilateral reduced grey matter concentration. The COWAT is thought to relate to frontal lobe dysfunction rather than specific temporal lobe damage. Therefore, association of decreased grey matter concentration in the hippocampal region is indicative of injury due to diffuse injury in the brain.

Although cognitive functions such as visual-motor integration, mental processing speed and verbal fluency are not specifically related to the hippocampal region, the fact that atrophic tissue changes were found in this region is suggestive that the hippocampal region, especially with regard to grey matter, is vulnerable to diffuse injury.

Hippocampal Region and Injury Severity

The Glasgow Coma Scale (GCS) is particularly discriminatory of diffuse brain injury. The present findings showed that decreased left hippocampal grey matter concentration at 1 and 6 months post injury was associated with GCS at A&E, which is supported by past research that has shown hippocampal size in head injured patients to be related to GCS (Tate & Bigler, 2000; Bigler et al., 1997). These correlations further suggest that atrophic changes in the hippocampus are likely to be

caused by diffuse injury to the brain rather than specific damage to the hippocampal region.

Limitations

For VBM to be successful there are a number of assumptions which must be met to ensure validity. It is crucial that segmentation occurs accurately and any confounding effects between groups must be eliminated or modelled as best as possible. Thus, effects that need to be taken into consideration include scanning patients and control subjects using the same scanner and the same MR sequences and also demographic variables such as age, gender and medication. In the current study, patients and controls were scanned on the same scanner using the same scanning sequence. There were no differences between the groups with regard to age and gender despite the control group having a greater ratio of males: females. Statistically, VBM assumes that the data is normally distributed which is important for parametric tests. Non-parametric tests should be used if this assumption cannot be met in order to avoid invalid results (Ashburner & Friston, 2000).

Segmentation of the brain into the 3 tissue classes relies largely on intensity values and employs a mixture model cluster analysis technique which assumes that MR images contain different tissue types and each voxel contains only one of these tissue types. A set of similarly normalised prior probability maps is used to specify the likelihood that each voxel belongs to one of the tissue types. Partial volume effects, which are confounded with larger voxel dimensions, can prove to be detrimental to successful segmentation making it essential that resolution of images is high, 1mm or

1.5mm isotropic voxels. Although the current study used a high resolution of 1mm, some anomalous results were returned; digit span and Trail Making Tests at 6 months produced results that were in the wrong direction. This may be explained by the inaccuracy of VBM to segment grey and white matter where there is poor grey/white matter differentiation. The hippocampal region and the hippocampus in particular, is one area of the brain where differentiation between grey and white matter is difficult. This inability to accurately differentiate between tissue classes has also been demonstrated in the brainstem and thalamus using an optimised VBM protocol (Good et al., 2001).

The pre-processing steps of VBM can vary between studies resulting in different researchers using different templates for normalisation, different smoothing kernels, different cluster extent thresholds and p values. Differences between templates used for normalisation have been demonstrated (Senjem et al., 2002; Good et al., 2001) and often, study-specific templates are created by normalising and smoothing all images and then averaging the images to produce a template. In a VBM study of schizophrenics conducted by Job and colleagues (2002), the original SPM99 T1 template was used in comparison to a study-specific template constructed from images of age-matched controls scanned on the same scanner as the patient group. Although the subject-specific template was found to detect more abnormalities, compatible results were obtained using both templates (Job et al., 2002). Furthermore, in a study using patients with temporal lobe epilepsy, hippocampal atrophy was detected regardless of the template used (Keller et al., 2004). However, study-specific templates may still be useful in studies where the standard template

may be inappropriate such as investigations using children (Wilke, Schmithorst & Holland, 2002).

Previous research using VBM to investigate hippocampal grey matter changes in conditions such as Alzheimer's disease and epilepsy has used smoothing kernels varying in size from 8mm – 12mm (Rombouts et al., 2000; Keller et al., 2004; Testa et al., 2004). However, it has been suggested that larger smoothing kernels reduce the ability of VBM to detect hippocampal grey matter; in a study of Down Syndrome, a smoothing kernel of 4mm but not 12mm detected reduced hippocampal grey matter that had been broadly reported in the Down syndrome literature (White, Alkire & Haier, 2003). The smoothing kernel of 10mm used in the current study was appropriate for analysis of the head injured population as results produced for age analysis and neuropsychological testing were supported by results from previous head injury studies.

In addition to the standard VBM method used in the current study, an optimised protocol has been developed which includes automated removal of non-brain tissue and additional spatial processing steps to improve image registration and segmentation (Good et al., 2001). The optimised procedure involves segmenting the images in native space and the resulting grey and white matter images are spatially normalised to respective grey and white matter templates to determine the optimised normalisation parameters. These parameters are applied to the original whole-brain images before further segmentation. The optimised method often includes an extra step to preserve the actual volume within a voxel. This modulation step is applied when analysing actual tissue volume and involves multiplying voxel values in the

segmented images by the Jacobian determinants (deformation parameters) obtained from spatial normalisation (Ashburner & Friston, 2000; Good et al., 2001).

In a study conducted by Keller and colleagues (2004), comparisons of grey matter concentration using both standard and optimised VBM and grey matter volume using optimised VBM was performed on patients with temporal lobe epilepsy. The images had been previously analysed using manual measurements and hippocampal atrophy was known to be present (Keller et al., 2002). Although the optimised method for grey matter volume identified extrahippocampal abnormalities related to temporal lobe epilepsy as well as hippocampal volume reduction, both standard and optimised VBM methods correctly identified atrophy in hippocampal grey matter concentration consistent with previous results using manual measurements. Standard VBM also identified reduced grey matter concentration in extrahippocampal regions that were previously not found to be abnormal using region-of-interest and histopathological studies (Keller et al., 2004). These results suggest that when analysing grey matter concentration, standard VBM may be more sensitive to subtle changes in neuroanatomy than optimised VBM. The results also suggest that differences reported in the literature with regard to specific neuropathology in disease states may be attributable to differences between standard and optimised VBM and whether the data have been modulated or not (Keller et al., 2005; Mechelli et al., 2005).

Although the current findings were mainly compared with studies using other methodology such as region-of interest measurements, the comparisons are valid due to the similarities between the current findings and the findings from other head injury studies. Also, the plausibility of results can be assessed with respect to what is

known about the distribution of atrophy in head injured patients. It has been noted that comparison between manual measurements and VBM is difficult due to each being judged by a different set of criteria (Geuze, Vermetten & Bremner, 2005). However, comparison with research employing techniques other than VBM was unavoidable due to the deficiency of VBM analysis of head injury contained in the literature. Moreover, there have been several comparisons of VBM and manual measurements in the literature and will be discussed in more detail in the general discussion.

CHAPTER 8: DISCUSSION

This study has analysed neuropsychological data in relation to structural changes employing two different neuroimaging methods, voxel-based morphometry and region-of-interest methodology via manual tracing. VBM analysis using whole scan analysis and using hippocampal masks in relation to head injury is, to date, unique to this study and adds to the growing literature concerning neuroimaging and head injury. Detailed discussion of neuropsychological, ROI and VBM data can be found in the respective chapters. In this discussion, the current findings will be discussed in the context of other imaging modalities and methodology and results from the two current methods will be compared alongside results from the neuropsychological assessments. Conclusions derived from the current study will be offered and finally, directions for further research will be suggested.

Neuroimaging

Various imaging modalities have been used to investigate head injury. While CT and MRI visualise anatomical changes in the brain, PET and SPECT are employed to explore pathophysiological and functional sequelae related to head injury. CT and MRI are the most commonly used imaging modalities with head injured populations and it is generally accepted that MRI is superior for detecting the extent of injury due to higher resolution and increased visualisation of brain structures. Moreover, the fact that MRI is a non-invasive technique allows its use in longitudinal studies.

For anatomical analysis of the hippocampus, MRI is superior to other imaging modalities due to having excellent differentiation of grey and white matter. The current study used manual tracing, thresholding and VBM methodology to analyse the hippocampus and corpus callosum but there are other methods available such as stereology, semi-automated methods using extraction techniques employing deformable shape models (Shenton, 2002) and automated techniques such as automated elastic matching (Iosifescu et al., 1997). Manual tracing has been criticised for being labour intensive and having inevitable potential inter and intra-observational measurement errors (Ashton et al., 1997; Shen, 2002), properties which newer, more automated techniques strive to overcome. However, despite these criticisms, decades of study using this method have contributed to the wealth of knowledge regarding anatomical structures in normal and abnormal states. Thus, rather than being redundant manually derived measures can assist in validating newer, automated techniques.

Apart from the optimised method of VBM, other methods that attempt to minimise potential bias introduced by volume changes during the registration procedure include deformation based morphometry (DBM) and tensor based morphometry (TBM). The deformation fields obtained from spatial normalisation of images to the template contain information about the individual image shapes which can be used in DBM to analyse differences in the relative positions of brain structures, and in TBM to localise differences in the shape of brain structures (Ashburner & Friston, 2000). TBM is essentially an amalgam of VBM and DBM as it enables analysis of positional and magnitude changes locally and globally. For small-scale differences, TBM requires very high resolution deformation fields, which is computationally

expensive. Therefore, using VBM to identify small-scale differences in large patients groups is a pragmatic approach that is within reach of research facilities.

Region of Interest Methodology in Comparison to VBM

Debate continues as to appropriate imaging techniques and analyses for specific purposes. The approaches employed in the current study were practical and relatively simple to implement. Direct quantitative comparison is not possible between VBM and region-of-interest methods used in the current study because each method investigated different properties of anatomy; VBM analysed grey matter concentration and ROI analysed volume. However, results from automated and manual methods are not expected to be identical, just as the ROI-based approach would not produce identical results if a different parcellation scheme were used. The main requirement when comparing ROI and VBM methods is that the results show the same general trends (Good et al., 2002).

Comparisons between VBM and ROI techniques have been carried out by Good and colleagues (2002) on temporal lobe structures in Alzheimer's disease and semantic dementia. Here, VBM detected a similar trend of atrophy to that found by an ROI method although VBM was found to perform better in the detection of hippocampal atrophy and ROI analysis was superior for the amygdala and temporal gyri (Good et al., 2002). Larger posterior hippocampi in London taxi drivers relative to controls have been corroborated using VBM and ROI (Maguire et al., 2000), as has grey matter concentration in temporal lobe epilepsy (Bernasconi et al., 2004; Keller et al.,

2002). Others have found VBM to be more accurate than ROI methods for detecting hippocampal atrophy in mild to moderate Alzheimer's disease (Testa et al., 2004).

As mentioned previously, a direct comparison is not possible but the determination of trends between the two methods is certainly viable. Age effects were found in the corpus callosum using thresholding and whole scan VBM and although manual tracing of the hippocampus failed to detect age effects, VBM analysis using masked hippocampal images showed a decrease in grey matter concentration with increasing age. The results for the corpus callosum illustrate how VBM and ROI analyses can compliment each other. Although age effects were found in the corpus callosum with ROI, additional data from the VBM whole scan analysis, illustrated that this effect was localised in the genu of the corpus callosum.

Information gained from association of neuropsychological data with grey matter concentration using VBM can be used to make parallel assumptions pertaining to association of neuropsychological data with anatomical volume gained from ROI analysis. Using MR images masked specifically for the hippocampus is novel and in the current head injured study, was considered an exploratory investigation. VBM associations of grey matter concentration and neuropsychological data were in the expected direction for nine cognitive tests namely, story recall, digit span, the Grooved Pegboard, BVRT, COWAT, Rey Figure, SDMT and Simple and Choice decision times. Thus, VBM analysis generally indicated that improved test performance was reflected in patients having greater grey matter concentration in the hippocampal region.

Although corresponding region-of-interest analysis did not return significant results for each cognitive test, results that were significant (Grooved Pegboard at 1 and 6 months and story recall at 6 months) were in the expected direction and mirrored VBM analyses by indicating that impaired patient test performance was associated with reduced hippocampal volume. Analysis of data from cognitive tests performed at 1, 3 and 6 months after injury implies improvement over this time period which is compliant with the ROI data that established no gross reduction in hippocampal volume or callosal area in this 5 month period. This within-group improvement over time may have been partly due to practice effects. A decrease in signal from 1 to 6 months was located in the frontal lobes when using whole scan analysis and reduced grey matter concentration was found in the parahippocampal gyri when using masked data in comparison to VBM controls. Injury severity as measured by the GCS at A&E was found to correlate with the left hippocampus at 1 month using ROI and at 1 and 6 months using VBM analysis. Collectively, the evidence regarding associations of hippocampal volume and grey matter concentration with cognitive testing and also with injury severity as measured by the GCS, provides mutual support for ROI and VBM approaches.

In the current study, VBM methodology using grey matter concentration was apparently more sensitive with regards cognitive testing and detecting age effects in the patient group. However, there may be some reservations regarding the reliability of the segmentation algorithm. Although lesions are known to be problematic in VBM, none of the patients exhibited lesions in the hippocampal area. The technique of masking for the hippocampus also avoids the effects of lesions and can be used for other structures in the brain. In terms of practicality, VBM is less time consuming

than manual tracing of brain regions. However, these apparent advantages do not necessarily mean that manual ROI approaches are redundant. VBM has shown consistency with ROI methodology in schizophrenia which also identified differences between patients and controls in the anterior cingulate. This was not identified by ROI as this structure was covered by a larger ROI analysis of the prefrontal lobe (Job et al., 2002; Job et al., 2003). Due to VBM analysis allowing regional comparisons throughout the entire brain, it can prove useful for suggesting possible abnormalities in structures not previously considered by ROI methods. This exploratory role of VBM enables structures to be identified and further explored and validated by ROI methods.

Region-of-interest methods are still classed by many as the 'gold standard' of measuring brain anatomy. However, there is a need for accurate and automated techniques which can eliminate potential rater bias and provide fast reproducible analysis. Given the rapid advances in computational neuroimaging, it may only be a matter of time before fully automated techniques are commonplace and consign ROI methods to the annals of history. Until that time, both VBM and ROI methods have complementary parts to play and will continue to be important tools in the clinical setting allowing the structure and function of brain disorders and their treatment to be analysed in an objective and quantifiable manner.

Neuroimaging and Mild Head Injury

With the increasing sensitivity of imaging modalities, more structural lesions are being detected in head injury in general and mild head injury in particular. The

majority of head injured studies have used moderately and/or severely injured populations and fewer studies have specifically investigated mild head injury despite 90 % of head injuries in the UK being classed as mild (Kay & Teasdale, 2001). Those that have concentrated on mild head injury have found significant numbers with abnormal scans (such patients are often referred to as having complicated mild head injury). Diffuse axonal injury lesions have been identified in 30 % of mild head injured patients using MRI (Mittl et al., 1994) and a majority of patients have been found to have abnormal MRI or SPECT findings with brain atrophy after 6 months suggestive of secondary ischaemic brain damage (Hofman et al., 2001). As the majority of the current patient group had abnormal MR findings, and required surgery, the group is representative of complicated mild head injury rather than typical mild head injury with no visible pathology. However, for the purposes of this study, 77 % of patients were classed as having mild head injury in accordance with the Glasgow Coma Scale at A&E.

Neuropsychological investigation of mild head injury has generated conflicting results in the literature. The interpretation of such results can be confounded by the use of different imaging modalities, cognitive tests, patient groups, time between assessments, levels of injury severity and diagnostic criteria. Although cognitive deficits have been reported in mild head injury, studies regarding mild head injury with abnormal scans are fewer in number and less clear. Weak correlation has been found between MRI abnormalities and reduced cognitive performance (Hofman et al., 2001) and marginal attentional differences have been found between patients with and without abnormal scans (Hughes et al., 2004). Although not a deliberate intention of the present study, the majority of the patient group had abnormal MR

images and it is worth taking account of this when considering the findings of neuroimaging and cognitive ability as such patients have been reported to have worse 6-12 month outcomes than those with normal scans and are more similar to patients with moderate injury (Van der Naalt et al., 1999b; Williams, Levin & Eisenberg, 1990).

Relationships with neuropsychological tests in the current patient group were identified in VBM analyses and to a certain extent in ROI analyses and are consistent with findings in the literature gained from mild to severely injured populations that is, neurocognitive impairment is found in head injury irrespective of injury severity. This is also supported by the comparisons of patient and orthopaedic control cognitive test scores at 1, 3 and 6 months post injury. Scores for story recall, digit span, BVRT, the Grooved Pegboard and the Trail Making Test B differed between the two groups providing further evidence that the patient group had suffered neurocognitive impairment as a result of head injury. Meta-analyses have concluded that cognitive impairment across a variety of domains is present in the first few weeks of mild head injury but baseline levels of functioning are resumed within 1-3 months (Belanger et al., 2005; Schretlen & Shapiro, 2003). However, with incomplete and deliberate exclusion of complicated mild head injury data respectively, these results reflect the sequelae of mild head injury without abnormal scans. A recent review by Ruff (2005) offered the conclusion that the focus on diagnostics has superseded the focus on treatment for head injured patients that present cognitive, physical and emotional symptoms after expected recovery (Ruff, 2005). The frequency and severity of long-term sequelae has been illustrated in a large study of 2,962 patients admitted to hospital with 90 % classified as having mild

head injury. After 1 year, 47 % of these patients had moderate or severe disability (Thornhill et al., 2000).

The recognition that mild head injury is accompanied with a high incidence of morbidity has led to suggestions that MRI be used following any head injury (Voller et al., 2001). In view of abnormal findings in imaging and cognitive deficit in mild head injury, consideration must be given to therapeutic intervention and rehabilitation of such patients which, at present, is considerably less than that offered to patients with moderate or severe head injury. A study has shown that patients who were assessed 1 week after injury and given an information booklet outlining symptoms associated with mild head injury and coping strategies, reported less symptoms and were less stressed after 3 months than a control patient group (Ponsford et al., 2002). Although it is apparent that sufferers of mild head injury are subjected to some cognitive difficulties, the nature and course of cognitive recovery remains controversial. The current findings provide further evidence of cognitive deficits in a mild head injured population where the majority of the group had abnormal scans. With evidence suggesting that mild brain injury may have a synergistic effect on the increased risk of Alzheimer's disease in patients with apolipoprotein-ε4 genotype (Mayeux et al., 1995), the increasing recognition of mild head injury as a major public health problem is crucial for the development of assessment and treatment procedures for such patients.

Head Injury and Neuroanatomy

The pathology of head injury is complex comprising focal injuries such as contusions and haematomas and diffuse injuries such as diffuse axonal injury and hypoxic damage. Processing of information is dependent on the integrity of neural structures and functional pathways that subserve a specific cognitive ability. Over the last decade, increasing neuroimaging advances has led to an increase in the number of quantitative studies using manual, semi-automated and fully automated techniques on a variety of brain structures. The hippocampus and corpus callosum have been previously quantified in both normal and abnormal states.

Long coursing white matter tracts are particularly vulnerable in head injury and atrophy of the corpus callosum has been previously demonstrated in head injury (Tomaiuolo et al., 2004; Johnson et al., 1996; Levin et al., 1990a). Atrophic changes in the hippocampus, which is also vulnerable after head injury, have also been previously demonstrated (Tomaiuolo et al., 2004; Tate & Bigler, 2000; Bigler at al., 1997). Whereas VBM identified decreased grey matter concentration in the hippocampal region, no atrophic changes were found in the current study using ROI methods. However, such absence of visible pathology does not necessarily equate to an absence of abnormality. As mentioned previously, ROI methods are insensitive to pathological conditions such as reductions of particular metabolites which can be detected by other imaging modalities such as magnetic resonance spectroscopy. Moreover, with degree of atrophy attributed to injury severity (Tate & Bigler, 2000; Gale et al., 1995), the nature of the head injury within the current patient group may reflect the lack of gross anatomical changes measurable by ROI.

The prefrontal cortex in humans has undergone more evolutionary development than any other species. The frontal lobes are thought to be involved in executive functions governing mood, behaviour and cognition and evidence from neuroimaging and neuropathology has established that such functions are compromised after head injury (Mattson & Levin, 1990; Levin & Kraus, 1994). Orbitofrontal and anterior temporal regions are particularly vulnerable in head injury due to their proximity to adjacent bony protrusions. Using a whole scan VBM technique, a reduction in signal was observed in the current patient group in the frontal lobes and anterior cingulate. Posterior cingulate atrophy association with injury severity has been previously demonstrated by Yount and colleagues (2002) using ROI methods. ROI methods may not be the most suitable for measuring this structure as anatomy between individuals can vary; patients may possess either a single gyrus or double parallel gyri which, in the case of the latter, can lead to greater surface area of the anterior cingulate. This may have accounted for injury severity association with posterior but not anterior cingulate gyrus (Yount et al., 2002). VBM methodology is one possible solution to the anatomical problem of single and double cingulate gyri and may prove to be more efficient at providing evidence of atrophy after head injury in this region.

While lateral prefrontal cortex damage has been linked with impairment to executive functions such as cognitive control, attention and working memory, orbitofrontal damage has been mainly characterised by social deficits. Beer and colleagues (2006) investigated social behaviour in patients with orbitofrontal damage, patients with lateral prefrontal damage and controls and found that those with orbitofrontal damage exhibited inappropriate social behaviour in an interpersonal task. The

patients only realised their behaviour was inappropriate after watching a videotape of their performance suggesting that damage to this region also impairs self-awareness (Beer et al., 2006). Fourteen months post injury, a patient with orbitofrontal damage was shown to have generally normal performance on cognitive tests such as story recall, the Rey figure and digit span but demonstrated deficits in social cognition (Cicerone & Tanenbaum, 1997). This implies that neuropsychological testing may be insufficient to fully identify psychological deficits in the head-injured patient; deficits due to orbitofrontal damage may impact on the patient's ability to interact in complex social behaviour while leaving performance on cognitive tests intact.

Diffuse injury of the brain can produce a gamut of neuropsychological deficits involving memory, attention, executive functions, slowed information processing and changes in personality and behavioural traits (Levin et al., 1990b). The post-concussional syndrome refers to the persistence of somatic, cognitive and affective symptoms which may confound the recovery after mild head injury and are thought to have an organic basis (Lishman, 1988). Global brain atrophy is a hallmark of diffuse injury and direct measures of such atrophy include a decrease in total brain volume and an increase in lateral ventricular volume (Blatter et al., 1997).

The current study provides evidence of diffuse damage in a group where the majority had complicated mild head injury. Despite a lack of gross structural changes in the corpus callosum and hippocampus in the 5-month period, reduced grey matter concentration was evident in the hippocampal region when compared to the VBM control group and cognitive test performance was associated with reduced grey matter concentration in the hippocampal region and to a lesser extent, with ROI

measurement of the hippocampus. The assumption that the hippocampus is inclusively involved in the cognitive processes assessed by the current test battery is over simplistic. Furthermore, a number of tests in the battery did not assess hippocampal function but were more suited to frontal lobe function; for example, digit span. The finding that such tests were associated with a reduction of grey matter concentration in the hippocampal region is suggestive of diffuse brain injury. This is strengthened by the whole scan VBM finding of reduced signal in the frontal lobes and associations of GCS score with cognitive data. These results suggest that diffuse damage disrupts the neural circuitry of different processing systems and hippocampal atrophy relating to cognitive deficits is a result of diffuse injury and generalised atrophy.

Varying neuropsychological performance has been demonstrated between patients with either focal or diffuse head injury (Wallesch et al., 2001; Wilson et al., 1995). Due to the majority of the current patient group having abnormal MR scans, it may be argued that damage caused by focal injuries confounded investigation between neuropsychological assessment and brain anatomy. Such an argument would continue by claiming that the effect of focal damage may have been responsible for the deficit in a particular cognitive domain and confounded relationships with volume change and grey matter concentration in specified brain structures.

Despite several patients displaying one or more abnormalities, it can be asserted that the current patient group had suffered from the effects of diffuse injury. Evidence for this comes from the fact that in ROI analysis, there were no gross changes in hippocampal or callosal volume between scans. Thus, indicating that gross anatomy

was not affected by either focal or diffuse injury. Furthermore, analysis of grey matter concentration was performed on images which had been masked to exclude all brain except the hippocampal region. This method, along with visual inspection of the hippocampal region which determined the absence of focal damage in this region, ensured that focal lesions would not be problematic and confound the data. Moreover, VBM whole scan analysis identified signal change in other parts of the brain, notably the frontal lobes. Therefore, although there may be cognitive differences in relation to focal or diffuse injury, cognitive deficits apparent in the current patient group are likely to be due to diffuse damage of the brain. With paucity in the literature regarding head injury and VBM, the current research has provided information regarding frontal lobe damage and diffuse damage to the hippocampal region.

Limitations and Further Research

With regard to patient sampling, the demographics of the current patient group were similar to those of other studies. The patient group was typical in age range (16-66 years), cause of injury and injury severity i.e. patients were predominantly mildly head injured as assessed by the GCS as is usual for patients who report to A&E departments with head injury (Kay & Teasdale, 2001). However, despite this mild classification, the patient group were a predominantly complicated mild head injury group typical of neurosurgical samples. The gender ratio of the patient group was dominated by males. Although this is typical for a city-based head injured sample group, it limited the current study to analysing the group as a whole rather than additionally determining any gender-specific differences which may have been

attributable to head injury. Further research with the inclusion of a greater number of female patients would allow for any gender-specific neuroanatomical differences to be ascertained and also anable gender analysis of cognitive and neurobehavioural deficiencies post head injury. As the patient sample was drawn from a large city region, it may be argued that results from such samples are biased and cannot be extrapolated to the general population. However, the results are empirically reliable in that they can be compared with other head injured studies, the vast majority of which have patient samples drawn from urban rather than rural areas. Another sampling issue may be that the patient population was entirely recruited from a single institution. Although recruitment of a multi-centre sample to provide a more diverse range of the head-injured population is preferable, it is not realistic in the majority of cases.

Whereas VBM analysis detected reduced signal and grey matter concentration between the patient and control groups, ROI analysis did not detect any structural changes within the patient group itself. However, absence of gross structural changes does not denote absence of changes within the patient group at the biochemical level. It may be that in the current study, structural MRI was not sensitive enough to convey the true extent of neuropathological damage. However, the results obtained from VBM seem promising. Diffusion tensor imaging is an MRI technique that is sensitive to white matter changes as it measures directional water diffusion in space which is determined by the integrity of myelin sheaths in white matter. Reduced axonal diffusion anisotropy in several white matter structures has been demonstrated in a mild head injured population in comparison to a control group (Arfanakis et al., 2002) and white matter changes have been shown to correlate

with injury severity and functional outcome (Huisman et al., 2004). Further research into head injury and the corpus callosum could utilise the technique of diffusion tensor imaging to investigate changes in this white matter structure as a result of trauma. It would be particularly suited to longitudinal analysis of head injury as it is a non-invasive technique.

Magnetic Resonance Spectroscopy generates maps of metabolic distributions by acquiring spectra simultaneously over a wide brain region and, as with diffusion tensor imaging, can identify neuropathology in brain regions which would be undetectable with conventional neuroimaging techniques. Metabolic changes of N-acetyl aspartate, a marker of neuron density and metabolism and may reflect axonal injury, has been detected in the splenia of mildly head injured patients (Cecil et al., 1998). Reduced N-acetyl aspartate has also been found alongside increased choline and myo-inositol that is suggestive of glial cell proliferation, in the frontal lobe of head injured patients (Garnett et al., 2000). Such studies provide evidence that a change in particular metabolites may be an indicator of neuropathological conditions in head injury which cannot be detected by structural MRI. The technique of magnetic resonance spectroscopy is suitable for longitudinal analysis due to its non-invasive nature and could be utilised in future research to investigate changes in metabolites in the corpus callosum and hippocampus after trauma.

There is also the possibility that changes in shape may have occurred which cannot be assessed using region-of-interest and VBM methods. Techniques which measure shape can be sensitive to changes in abnormal brains where other measures are not. This was demonstrated in a schizophrenic patient group where no change in callosal

area was evident in relation to controls but a shape measure using a surface deformation-based approach discriminated between the two groups (Narr et al., 2000). Hippocampal shape descriptors using high-dimensional brain mapping have also proved more effective than volumetry in discriminating schizophrenic patients from controls (Wang et al., 2001). Others have argued that each approach provides complementary information (Gerig et al., 2001). Longitudinal shape analysis of the hippocampus and corpus callosum post head injury would provide valuable information and would be particularly useful to further investigate patient groups such as the current one where region-of-interest analysis did not demonstrate any volumetric changes. Changes in the shapes of neuroanatomical structures may reflect changes in neural architecture which cannot be ascertained by volumetric measures. Such shape changes can consequently affect neural connectivity to and from these structures and in turn, affect functionality.

Focal lesions are problematic in the pre-processing steps of VBM analysis resulting in image distortions and imprecise segmentation which have obvious detrimental effects in subsequent statistical analysis. Although abnormalities can be masked to avoid such problems, doing so for large cohorts containing a large number of lesions is time consuming and does not fit the ethos of the neuroimaging community that is striving to achieve precise fully automated methods. Whole scan analysis (Stamatakis & Tyler, 2005) is a viable alternative and using this method, signal changes in appropriate brain regions were identified in the current patient group. To the author's knowledge, this is the first VBM study to use a predominantly mild head-injured population and the first to use whole scan analysis and hippocampal-masked images with regard to head injury. Time restraints were such that whole

scan analysis with neuropsychological data was not possible. Further VBM research of head injury would benefit by using whole scan analysis to investigate possible relationships with neuropsychology. This data would complement the results found with the hippocampal-masked images and provide further insight into neuropsychological impairment and diffuse brain injury.

As the apolipoprotein-ε4 allele has been associated with smaller hippocampi in cognitively normal subjects and those in the early stages of Alzheimer's disease (Bigler et al., 2000), an informative adjunct to the current study would have been to investigate genotype with regard to hippocampal volume, VBM analyses and neuropsychological data. Such research would have contributed to the literature concerning head injury and genotype by providing data from a predominantly mild head-injured patient group. To date, there is no VBM analysis that has investigated genotype with regard to head injury. Such investigations, with their increased sensitivity and ability to process large numbers of patients relatively quickly, would provide valuable data for the study of head injury. Although the current patient group had been previously genotyped for apolipoprotein-ε4, the research protocol determined that these analyses could not be undertaken.

Conclusions

In the current study, VBM analysis demonstrated differences in signal change and grey matter concentration between the patient and VBM control groups and reduced cognitive performance was associated with grey matter concentration in the hippocampal region denoting diffuse brain damage. VBM detected longitudinal

frontal lobe changes in the patient group but no changes were found in the hippocampus or corpus callosum and no longitudinal changes were found using ROI. Hippocampal relationships with injury severity were found using both VBM and ROI methodology. With regards neuropsychological assessment, the patient group showed improved cognitive ability from 1 to 6 months post injury indicating that some degree of cognitive recovery had occurred; this was complemented by association of cognitive ability with injury severity and cognitive differences between patient and orthopaedic control groups. Despite this apparent recovery of cognition, neurobehavioural deficits were evidenced by the substantial number of patients reporting symptoms of depression and anxiety and by the majority of patients failing to resume work and social activities at 6 months post injury.

General trends were apparent for agreement between the two methodologies employed. ROI and VBM both identified age effects in the corpus callosum and both identified associations with neuroanatomy and cognitive impairment in immediate story recall and Grooved Pegboard using the non-dominant hand. However, VBM analysis was apparently more able to detect relationships between neuropsychological impairment and neuroanatomy. Of the 22 cognitive tests administered at 1 and 6 months post injury, VBM had 12 associations in the expected direction while ROI had 5 associations. VBM was also more sensitive to injury severity as measured by the GCS at A&E; a reduction in grey matter concentration was associated with injury severity at both 1 and 6 months whereas ROI hippocampal volume was associated with injury severity at 1 month only. Despite the apparent sensitivity of VBM analyses, the current study also demonstrated segmentation issues regarding VBM analysis of white matter and CSF. Therefore

although VBM is a promising technique for investigation of head injury, segmentation issues need to be addressed.

The VBM methods used here, whole scan analysis and masked hippocampal regions, have not previously been used to investigate head injury. Using these methods, evidence of the effects of diffuse injury was detected in the frontal lobes, cerebellum and hippocampal region. Furthermore, VBM analysis using masked images was sensitive to relationships with cognitive impairment at 1 and 6 months post injury. Therefore, VBM may be employed for exploratory analysis or to study specific regions in head injury. These techniques, in alliance with ROI volumetry and imaging modalities sensitive to biochemical disturbances, can facilitate an understanding of the neuropsychology of head injury and the neuropathology of general atrophy and the specific structural atrophy that underlies it. Advances in neuroimaging analysis can expand knowledge of the psychobiology of head injury and provide for the development of assessment and treatment procedures to ensure the most favourable outcome for such patients.

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