Traumatic Brain Injury:

Long-Term Neural and Cognitive Outcomes

By

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Dedication

This dissertation is dedicated to my ever loving and supportive husband Jeremy Bryce Miller Farbota. During my time as a graduate student he has provided unwavering encouragement. He has been my sounding board, my caretaker, and my anchor. He has been cheerfully willing to pick up the slack when the rigor of my graduate studies left me unable to fulfill the basic obligations of a functional adult human. Without him the sane and timely completion of this dissertation would not have been possible, and I am inexpressibly grateful.

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Abstract

Traumatic brain injury (TBI) is the most common cause of disability among young adults, and a single traumatic event may have lifelong implications. This dissertation begins by providing a background on the current understanding of the systemic and cellular level understanding of the TBI disease state. Next, three scientific studies are presented. Each assesses a different aspect of longitudinal TBI-induced brain change by following a cohort of TBI patients for a four year period post-injury. The first study uses diffusion tensor imaging to assess how white matter damage progresses, and whether white matter change has neuropsychological correlates. Results indicate that while some brain regions, such as the corpus callosum, show continued longitudinal degeneration for several years following an injury, other regions exhibit longitudinal improvements. Specifically, bilateral regions of the superior longitudinal fasiculus demonstrate significant increases in white matter integrity in patients, something never previously observed in human patients. The second study uses tensor based morphometry to identify regional patterns of volumetric contraction following TBI. Results indicate that TBI patients exhibit volume loss throughout the brain (both in gray and white matter) during the first year post-injury, and pervasive continued volume loss focused in brain white matter between the second and fourth years post-injury. The final study uses functional MRI to observe patient BOLD response to a memory encoding task. Results indicate that patient exhibit much greater variability in their patterns of activation than controls. Furthermore, patients with low levels of cognitive reserve tend to hypo-activate, while patients with more severe injuries tend to hyper-activate to the task. Four years post injury, all patients ages 28 and under demonstrated normalized patterns of activation while none of the patients ages 29 and over exhibited normalized BOLD responses. Taken together, these studies indicate that TBI should be treated

not as an isolated incident, but as the initiation of a disease state. The final chapter discusses how these three studies relate to one another, what their clinical implications may be, and what future directions may be best for continued study of the TBI disease state.

Chapter 1:

Introduction

Background

Despite the high incidence and prevalence of traumatic brain injury (TBI), very little is known about the long-term neurological progression of this acquired disease. In order to better treat patients suffering TBI-induced disability, it is first necessary to further our understanding of the neurological underpinnings of TBI-related impairment. The purpose of this dissertation is to improve our appreciation of the neurological disease state underlying TBI in order to facilitate improved prognosis and treatment for TBI patients.

Epidemiology

Traumatic brain injury (TBI) is the most common cause of disability among young adults (van Baalen et al., 2003). The incidence of TBI is estimated to be over 1.5 million in the United States alone, and more than 5.3 million Americans are living with TBI-related impairment (Langlois et al., 2006; Rutland-Brown et al., 2006). All studies of TBI incidence in the United States find that TBI is roughly twice as common among males compared to females, and most common among individuals aged 15-24 ("Incidence rates of hospitalization related to traumatic brain injury--12 states, 2002," 2006; Jager et al., 2000). Annual injury rates are also known to be higher among lower income individuals and persons with lower levels of educational attainment (Silver et al., 2005). Some of the most common etiologies of moderate to severe TBI include motor vehicle accidents, falls, and assaults (Riley et al., 2004). Motor vehicle accidents, particularly those involving motorcycles, are by far the most common cause of TBI (Weiss et al., 2010).

Neuropathology

TBI is characterized by both coup and contra-coup at impact (the brain hitting first the part of the skull making impact and then the opposite side of the skull as the head recoils) and by the shearing and tearing or axons throughout the brain due to rotational acceleration of the head. Common diagnoses associated with TBI include skull fracture, concussion, hemorrhage, hematoma, contusions, lacerations and focal lesions (Silver et al., 2005). In addition, secondary pathologies, including further hemorrhage and hematoma, mechanical deformation of cells and raised intracranial pressure can occur as complications following initial injury (Bigler & Maxwell, 2011). Over time, it is known that TBI patients exhibit gross brain volume loss that progresses for at least one year post injury (Bendlin et al., 2008; Sidaros et al., 2009; Trivedi et al., 2007). Furthermore, studies show that brain white matter, the tissue composed of axonal tracts connecting regions of cortex, is harmed in TBI (Ding et al., 2008; Gale et al., 1995). Other complications include vascular disruptions and injury to cranial nerves (Golding, 2002).

In addition to the gross pathology, understanding the cellular level pathology of TBI is critical for developing new treatments for TBI. Neurochemical changes that follow TBI likely include both "neuroprotective" and "autodestructive" agents (Silver et al., 2005). The timing of neurochemical changes following injury can provide a window of opportunity for treatment. Immediately following injury acetylcholine increases are observed (Donat et al., 2008; Lyeth & Hayes, 1992). Circulating epinephrine and norepinephrine levels increase with the severity of the TBI, and levels of serotonin and dopamine are thought to increase regionally surrounding the site of injury (Dunn-Meynell et al., 1998; Goldstein, 2003; Kobori et al., 2011). However, it is not well understood how these initial neurochemical cascades affect long-term outcomes. In the days and weeks following injury, increases in neurotrophic factors, such as nerve growth factor (NGF)

and fibroblast growth factor (FGF) are thought to promote recovery (Chiaretti et al., 2008; Mellergard et al., 2010; Ziebell & Morganti-Kossmann, 2010).

Autophagy, a process by which the brain removes dead or damaged tissues allowing remaining healthy cells to function more effectively, is also thought to promote recovery (Clark et al., 2008; Liu et al., 2008; Y. B. Zhang et al., 2008). However, concurrent encephalopathy is known to lead to necrosis and apoptosis throughout the injured brain which portends continued Wallerian degeneration for an unknown amount of time post-injury (Denecker et al., 2001; Kelley et al., 2006; Raghupathi, 2004; X. Zhang et al., 2005; Zhou et al., 2012). Wallerian degeneration, during which axons damaged by shearing and tearing at the time of injury degenerate toward the cell bodies, is a pervasive part of TBI pathology (Kelley et al., 2006). This process likely contributes to the white matter change observed in TBI, and may play a role in both degeneration and reorganization. Co-occurring ameliorative and degenerative processes create a complex brain environment that varies dramatically between TBI patients. Further research is needed to understand how different cellular processes interact in the long term, and whether neurochemical imbalances and progressive cellular changes should be investigated as therapeutic targets.

Symptoms and Co-Morbidities

Symptoms associated with moderate to severe TBI are numerous and varied. Emotional symptoms often include depression and anxiety (Morton & Wehman, 1995; Rapoport, 2012). These symptoms can range greatly in duration and severity, but are often treatable with pharmacotherapy (Alderfer et al., 2005). Cognitive impairments include attention deficits, slower speed of cognition, and most commonly, memory problems (Clune-Ryberg et al., 2011; Kim et

al., 2009; Vakil, 2005). Cognitive impairments in TBI patients often demonstrate some degree of recovery over time, but particularly in moderate to severe injuries, patients rarely regain their pre-injury level of functionality (Catroppa & Anderson, 2002; Jeon et al., 2008; Marquez de la Plata et al., 2008). Very long term studies of TBI have indicated that memory problems are the most consistent complaints that affected individuals voice (Brown et al., 2011). TBI patients also demonstrate discrete behavioral problems, such as aggression, irritability, and blunted social intelligence (Crowe et al., 2012; Diaz et al., 2012; Yang et al., 2012). TBI patients also frequently experience motor impairments, fatigue and sleep problems, headaches, balance problems and dizziness, vision impairments, chronic pain and sexual dysfunction (Hoffer et al., 2007; Kiraly & Kiraly, 2007; Lucas et al., 2012; Ponsford et al., 2012). The exact constellation of symptoms experienced by an individual patient is determined by the precise regions of the brain that have experienced damage, either as a result of primary injury or secondary complications.

Traumatic brain injury is associated with increased risk for a variety of co-morbid disorders. These include major depressive disorder, anxiety disorders, and dementias later in life. Co-morbid depression is thought to affect up to 61% of TBI patients, and is associated with reduced cognitive and behavioral recovery (Rapoport, 2012; Seel et al., 2010). Anxiety disorders are also common and are associated with poorer recovery of independence and psychosocial maladjustment (Morton & Wehman, 1995). Seizure disorders and epilepsy are often observed following TBI (Zhao et al., 2012). Several studies have demonstrated that individuals sustaining a TBI early in life are significantly more likely to suffer from dementia later in life (Bazarian et al., 2009; Kiraly & Kiraly, 2007). In particular, Alzheimer's disease is significantly more common among individuals who have sustained a TBI than those who have not, regardless of

other factors (Van Den Heuvel et al., 2007). The high incidence of co-morbid neuropsychiatric diseases that occur in conjunction with TBI increases the clinical importance and magnitude of this building epidemic.

Treatments

Treatment options for traumatic brain injury include pharmacotherapy, psychotherapy, cognitive rehabilitation and behavioral treatments. In contrast to many other disease states, the variation in TBI patients means that the proper pharmacotherapy for individual patients varies considerably. Neuropsychiatric symptoms arising from focal trauma are understandably influenced by the location of focal injury, and so in turn are patient symptoms and ideal drug treatment courses. Some of the most common emotional symptoms experienced by TBI patients are depression and anxiety (Hynes et al., 2011). Compared to primary major depressive disorders, tricyclic anti-depressants (TCAs) are thought to carry higher risks and be less effective for TBI patients (Mahesh et al., 2010). Similarly, monoamine oxidase inhibitors (MAOIs) are also not frequently prescribed to TBI patients because the complex dietary restrictions these drugs necessitate may be difficult to comply with for individuals experiencing TBI-related cognitive impairment (Newburn et al., 1999; Sonawalla & Fava, 2001). Therefore, selective serotonin reuptake inhibitors (SSRIs) are the preferred anti-depressant treatment for TBI patients (Rapoport et al., 2008). Anxiety treatment in TBI patients also differs from treatment for primary anxiety issues. Benzodiazepines, for instance, may further impair motor and memory function and are therefore avoided in TBI patients (Silver et al., 2005). Busiprone, a partial serotonin agonist, is a better tolerated anxiolytic for TBI patients (Kline et al., 2012; Olsen et al., 2012). Cognitive impairment following TBI is also sometimes treated with pharmacotherapy.

Methylphenidate and other psychostimulants are sometimes prescribed to treat slowed speed of cognitive processing and attention impairment, while cholinesterase inhibitors are occasionally used to treat memory complaints (Tenovuo, 2005; Willmott & Ponsford, 2009). Given the variable nature of TBI, pharmacotherapy is patient specific and at this point in time it is used to treat individual symptoms rather than the disease as a whole (Wheaton et al., 2011; Writer & Schillerstrom, 2009).

In TBI drug treatment alone is often not enough to bring about meaningful improvements in patients' lives. Psychotherapy is an important compliment to pharmacotherapy. The goal of psychotherapy in TBI patients, as with any set of patients, is to facilitate the re-establishment of an acceptable sense of self, which can in turn alleviate symptoms such as depression and anxiety (Galante et al., 2011). Psychotherapy treatment of TBI patients differs from treatment of other patients in that a much greater level of therapist flexibility is required as these patients' progressing neurological injuries put them in a state of constant flux (Alderfer et al., 2005). Generally, psychotherapy is highly effective in TBI patients and an important part of the recovery process (Thurmond et al., 2010).

In addition to psychotherapy to treat emotional issues, cognitive rehabilitation is important for the treatment of specific impairments. In cognitive rehabilitation, neurological and neuropsychological evaluations are used to identify specific impairments, and rehabilitation strategies are then selected to meet patient-specific goals (Cernich et al., 2010; Cicerone et al., 2011). Exercises that improve memory capacity, extend the attention span and promote mental organization have all been found to positively influence general cognitive function in TBI patients (Cicerone et al., 2011; Dams-O'Connor & Gordon, 2010). However, "booster"

treatments are often necessary for patients to maintain gains made through cognitive rehabilitation (Cernich et al., 2010).

In addition to cognitive and emotional problems, TBI often causes discrete behavioral issues that lower patient quality of life. Behavioral impairments often include increased aggression, diminished self-care skills, and impaired interpersonal skills, all of which cause issues in the psychosocial domain of patients' lives (Benedictus et al., 2010). Behavioral treatments seek to address specific issues causing the greatest difficulties for individual TBI patients by helping patients to develop specific coping skills that replace maladaptive behaviors with adaptive behaviors (Truelle et al., 2010). This is frequently made difficult by a lack of awareness of impairments that many patients experience, however specifically targeted behavioral treatments have been shown to be effective in the treatment of TBI patients (Hart et al., 2009).

While the currently available treatments, particularly when administered in combination, help TBI patients to make significant gains, patients often never return to their pre-injury state of cognitive and emotional well-being (Levack et al., 2010; Levine & Flanagan, 2010). One reason patients may not be achieving their maximum possible level of recovery is that treatments are frequently only administered for three to twelve months post-injury, while there is reason to believe that the progression of TBI continues for several years (Masel & DeWitt, 2010). By developing a deeper understanding of the neurological changes that precipitate continued impairment, the development of more effective treatment courses will be made possible.

Current understanding of the TBI disease state includes significant research concerning the cellular processes that occur shortly after injury, and considerable knowledge of short-term global changes, co-morbidities, neuropsychological deficits and how treatment can improve

outcome. However, our understanding of the large-scale, long-term changes that occur in the brain following TBI, the processes that push the TBI brain into a new and different state that makes it distinct from the never traumatically injured brain for the remainder of a patient's lifetime, have not been longitudinally characterized. To address this paucity of information, a series of longitudinal neuroimaging studies that investigated several facets of global brain change over a multi-year period post-injury were carried out. Below, the specific aims and methods of each of these studies are summarized. This set of investigations was motivated by the need to understand how the TBI disease state develops in the month and years following injury and how individual variability contributes to outcome, with the hope that information gathered will have future clinical relevance.

Specific Aims, Hypotheses and General Overview

Specific Aim 1: Use diffusion tensor imaging to determine the duration and specific pattern of white matter change induced by TBI, and identify neuropsychological relevancies.

Diffusion tensor imaging (DTI) has previously been shown to be sensitive to TBI-induced changes in white matter microstructure. An important feature of TBI is that in addition to lesions caused by the initial coup and countercoup of impact, there is diffuse shearing and tearing of axons throughout the brain caused by rotational acceleration of the head. These diffuse lesions, sometimes referred to as diffuse axonal injury (DAI) are focused in brain white matter, and may represent as important a part of TBI pathology as the focal lesions. The hypothesis this specific aim tested was that white matter damage is present throughout the brains of TBI patients, and that white matter damage in TBI brains does not just persist but evolves and changes for several years following injury, demonstrating some degree of recovery alongside progressive degeneration. To test this, TBI patients and healthy controls underwent DTI at two months, one year and four years post-injury, and comparisons were made both between groups and across time points. Correlations between DTI results and neuropsychological task performance were also addressed.

Specific Aim 2: Use tensor based morphometry to determine the pattern of brain tissue degeneration following TBI and isolate correlations between brain volume loss and neuropsychological function.

Brain volume change following TBI has been examined both longitudinally during the first year post-injury and cross-sectionally in individuals whose injuries are several years to

several decades old. TBI patients consistently exhibit reduced brain volume compared to uninjured controls, and often specific tissue degeneration can be identified surrounding impact loci. Tensor based morphometry (TBM) is a process that determines how much stretching or contracting would be necessary to transform one three dimensional structural brain image into a second one. In longitudinal studies, this technique can therefore be used to derive maps identifying regions in which individuals have demonstrated increases or decreases in brain tissue volume between two scans. In light of surmounting evidence indicating that TBI is the initiation of an evolving disease process, it is necessary to develop further understanding of the progression of neurological change following these injuries beyond the first year post-injury. The hypothesis this specific aim tested was that brain volume loss continues beyond the first year post-TBI, and this volume loss relates to cognitive and other functional outcomes. To test this, TBM analyses were performed on the same cohort of subjects with three data time points described in Specific Aim 1 and brain volume change was analyzed during both the first interval (two months to one year) and the second interval (one to four years post-TBI) to determine both the extent and specific pattern of brain volume change. The relationship between neuropsychological measures and volumetric changes in TBI patients' brains was also assessed.

Specific Aim 3: Use functional magnetic resonance imaging to identify alterations in brain function following TBI and determine whether and how these alterations relate to patient characteristics and outcome.

Functional magnetic resonance imaging (fMRI) enables researchers to observe correlates of neuronal function in the active human brain. Previous studies have demonstrated that TBI patients, both the recently injured and to a lesser extent those considered in the chronic stage of

their injuries, demonstrate patterns of brain activity that are different from the patterns exhibited by healthy controls. Because TBI often results in damage to brain regions critical for encoding and retrieval of episodic memories, the task employed in this study focused on recognition of previously viewed objects versus novel objects. Psychologically, TBI patients demonstrate initial memory deficits compared to controls, however longitudinal fMRI tracing the course of changes in brain activity accompanying these changes in memory function have not been previously performed. The hypothesis tested by this project was that TBI patients would demonstrate patterns of activation that are different from those demonstrated by controls, and that patient characteristics could be used as explanatory variables and prognostic indicators of future functional status. To test this, fMRI data were collected from the same subjects as described in Specific Aims 1 and 2 to determine if TBI patients exhibit different patterns of activation compared to controls, and whether there are changes in the patterns exhibited by patients as they recover from their injuries.

General Overview

The following chapters include three articles (one assessing each of the above stated specific aims) that provide novel information about the progression of traumatic brain injury. The final chapter is a synthesis of these studies and compares the diagnostic and prognostic values of the three methods employed, discusses how the three projects complement one another, and puts forth ideas for further research. Taken together, these studies contribute to a growing consensus in the field of TBI research that contends that these injuries should be looked at not as isolated incidents, but as the initiation of a prolonged disease state. This new conceptualization of TBI has important clinical ramifications in the realm of long-term treatments for TBI patients.

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Chapter 2:

Longitudinal diffusion tensor imaging and neuropsychological correlates in traumatic brain injury patients

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Abstract

Traumatic brain injury often involves focal cortical injury and white matter (WM) damage that can be measured shortly after injury. Additionally, slowly evolving WM change can be observed but there is a paucity of research on the duration and spatial pattern of long-term changes several years post-injury. The current study utilized diffusion tensor imaging to identify regional WM changes in 12 TBI patients and 9 healthy controls at three time points over a four-year period. Neuropsychological testing was also administered to each participant at each time point. Results indicate that TBI patients exhibit longitudinal changes to white matter indexed by reductions in fractional anisotropy (FA) in the corpus callosum, as well as FA increases in bilateral regions of the superior longitudinal fasciculus (SLF) and portions of the optic radiation. FA changes appear to be driven by changes in radial (not axial) diffusivity, suggesting that observed longitudinal FA changes may be related to changes in myelin rather than to axons. Neuropsychological correlations indicate that regional FA values in the corpus callosum and sagittal stratum correlate with performance on finger tapping and visuomotor speed tasks (respectively) in TBI patients, and that longitudinal increases in FA in the sagittal stratum (SS), SLF and optic radiation (OR) correlate with improved performance on the visuomotor speed (SS) task as well as a derived measure of cognitive control (SLF, OR). The results of this study showing progressive WM deterioration for several years post-injury contribute to a growing literature supporting the hypothesis that TBI should be viewed not as an isolated incident but as a prolonged disease state. The observations of long-term neurological and functional improvement provide evidence that some ameliorative change may be occurring concurrently with progressive degeneration.

Introduction

Traumatic brain injury (TBI) affects more than 1.4 million people every year in the United States (CDC, 2006). These injuries are the most common source of neurological impairment among young and middle-aged adults, and can produce long-term cognitive deficits that hinder patients' ability to function independently, lower their quality of life and increase the risk of developing co-morbid neurological disorders (Anderson, Bigler, & Blatter, 1995; Bombardier et al., 2010; Kiraly & Kiraly, 2007; Malec, Brown, Moessner, Stump, & Monahan, 2010; Risdall & Menon, 2010; Sharp & Ham, 2011). Previous research has shown that the progression of structural pathology in the first year following injury includes decreased white matter integrity throughout the brain (Lin et al., 2010; Marquez de la Plata et al., 2008; Povlishock & Christman, 1995; Sidaros et al., 2008; Trivedi et al., 2007; Xu, Rasmussen, Lagopoulos, & Haberg, 2007), but little is known about white matter changes that occur after this first year. Concomitant to slowly occurring atrophy and WM degradation, TBI patients typically demonstrate measurable cognitive and motor improvements in the first and subsequent years post-injury. This study aims to identify long-term patterns of white matter change following TBI, as well as how variations in white matter integrity correlate with both neuropsychological test performance and change in test performance over time (Levin, 2003; Staudt, 2010).

Diffusion-tensor imaging (DTI) is sensitive to white matter damage immediately following TBI and useful in monitoring longitudinal changes (Bendlin et al., 2008; Filippi, Ulug, Ryan, Ferrando, & van Gorp, 2001; Xu et al., 2007). DTI, which is based on the principle that water molecule movement is restricted by barriers to diffusion that vary in the brain depending on tissue type or pathology, [for a review see (Le Bihan, 1991)], is sensitive to changes in the

microstructure of white matter. Several studies have shown that DTI accurately detects damage in tissue that may appear normal when measured with conventional MRI (Arfanakis et al., 2002; Chan et al., 2003) and that DTI can be of clinical importance when tracing recovery (Arfanakis et al., 2002; Field, Hasan, Jellison, Arfanakis, & Alexander, 2003; Filippi et al., 2001). These capabilities make DTI well suited for assessing white matter damage caused by TBI, and for tracking how white matter changes progress longitudinally following injury.

The current study is an extension of a prior study conducted on this same cohort of TBI patients. The previous paper, (Bendlin et al., 2008), included whole-brain DTI and volumetric analyses of patients at 2 months and 1 year post-injury. Results showed gray and white matter alterations over time in TBI compared to control. Regions of reduced white matter integrity included corpus callosum, forceps major and minor, anterior corona radiata, external capsule, cerebral peduncle, superior longitudinal fasciculus, uncinate fasciculus and corticopontine tract. The current study extends the previous work with the addition of a third time point approximately 4 years post-injury. Furthermore, the current study includes axial and radial diffusivity analyses (which may allow inference concerning the cause of white matter changes), and increases the power to detect group-wise effects by restricting analyses to the white matter.

The primary metric used to assess white matter integrity in this study is fractional anisotropy (FA). FA describes the extent to which a diffusion process is anisotropic, or directionally constrained. In brain white matter, higher FA is associated with greater white matter (Alexander, Lee, Lazar, & Field, 2007). As noted above, we also employed two secondary metrics: Axial diffusivity and radial diffusivity. Axial diffusivity refers to the movement of water along the principle axis of a white matter tract. Animal studies have demonstrated that high axial

diffusivity is associated with healthy axons, and low axial diffusivity is associated with axonal damage (Song et al., 2003; Song et al., 2002). Radial diffusivity refers to the movement of water perpendicular to the principle axis of a white matter tract. Animal studies have demonstrated that low radial diffusivity is associated with healthy myelin, and high radial diffusivity is associated with myelin damage (Wheeler-Kingshott & Cercignani, 2009). Investigating how these secondary metrics change over time in brain regions demonstrating longitudinal differences in FA can provide additional insight into the processes underlying white matter change.

The first major goal of the current study was to characterize longitudinal changes in regional brain white matter microstructure using DTI in conjunction with neuropsychological testing. Due to previous reports of volume loss and white matter decline in the corpus callosum, as well as the emergent theory of TBI as the initiation of a disease state, we predicted that this structure would demonstrate continued decline throughout the duration of the study within the TBI group (Bigler et al., 1996; Gale, Johnson, Bigler, & Blatter, 1995; Kim et al., 2008; Kumar et al., 2010; Ljungqvist et al.; Masel & DeWitt, 2010; Matsukawa et al., 2011; Wu et al.). While decline in many regions is likely, patients typically continue to improve cognitively; thus we also sought to determine whether FA increases may occur in other regions, which may suggest consolidation or remodeling of white matter tracts that are associated with recovery. Candidate regions include corticospinal tract regions (cerebral peduncle, internal capsule) and longitudinal tracts (superior and inferior longitudinal faciculi) due to previous research indicating improvements in certain diffusion metrics in a subset of these regions (Sidaros et al., 2008) and the presence of damage in these regions observed in cross-sectional studies done close to the

time of injury but not in those done several years post-TBI (Bendlin et al., 2008; Caeyenberghs et al.; Kraus et al., 2007).

The second major goal of this study was to identify correlations between FA and neuropsychological task performance cross-sectionally at each time point studied, as well as determine whether changes in task performance over time correlated with changes in FA longitudinally. Due to previous research indicating that higher corpus callosum FA is associated with better performance on manual motor tasks in brain injury patients, we predicted that scores on a fine motor finger tapping task employed in this study would correlate with FA in this region among TBI patients (Caeyenberghs, Leemans, Coxon et al., 2011; Caeyenberghs, Leemans, Geurts et al., 2011). Furthermore, we predicted that increases in FA within the longitudinal tracts would correlate with improved performance on the more complex neuropsychological tasks such as the Cognitive Oral Word Association Test (COWAT), or the cognitive component of the Trail Making Test. Finally, we expect patients who have sustained more severe injuries, as measured by the 24 hour post- resuscitation Glasgow Coma Score (GCS), to demonstrate a greater degree of initial microstructural damage and longitudinal white matter change than patients who sustained less severe injuries.

Methods

TBI patients

Forty-six TBI patients participated in an initial MRI scan, thirty-six returned for a second visit and twenty returned for a third visit. DTI was acquired in 16 individuals at all three time points (three were subsequently excluded due to excessive motion, and one more was excluded due to a second head injury sustained in a motor vehicle accident between visits two and three). The mean age of the final group of twelve patients (ten males and two females) was 35.00 ± 12.76 years at the beginning of the study; mean education was 13.17 ± 1.75 years. The majority of the patients received acute treatment at the University of Wisconsin Hospital and Clinics level 1 trauma center and were referred from the departments of Neurosurgery, Trauma and/or Rehabilitation. The inclusion criteria for TBI consisted of involvement in a rapid impact injury to the brain (such as a motor vehicle accident or fall) causing a loss of consciousness. Evidence of brain injury included admittance for emergency medical attention following loss of consciousness in the field, a Glasgow Coma Scale (GCS) score either at the emergency room (ER) or upon hospital admission of less than or equal to 13, and a post-resuscitation GCS score of 5 or above. All patients had day of injury CT scans that were positive for visible brain injury. All TBI patients were less than 3 months post-injury at their first visit, and most were studied between 8 and 12 weeks post-injury depending on their availability and other medical issues related to the injury. Exclusion criteria consisted of current major Axis I psychiatric disease or history of major medical condition (e.g. cancer, diabetes, or diagnosed neurological condition), as well as any previous diagnosis of substance dependence, or an undiagnosed pattern of behavior demonstrating longstanding maladaptive use of alcohol or other drugs. All patients

gave informed written consent under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board.

Healthy Controls

Thirty-six control participants were recruited from the community and from the University of Wisconsin Madison campus via advertisement. Twenty of these participants returned for two additional visits. Acquisition errors resulted in the loss of four participants who did not have adequate DTI at all three time points; and in seven cases DTI was not acquired at one of the visits due to time constraints; DTI scans acquired from nine participants at all three time points were used in the final analysis. The mean age of the final group of nine healthy controls (four males, five females) was 31.44 ± 12.38 years at the beginning of the study; education was 14.77 ± 2.22 years. Exclusion criteria were identical to the TBI group (with the exception of head injury). MR scanning of control participants occurred on approximately the same schedule as that of TBI patients. All participants gave informed written consent under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board.

Procedures

Volunteers participated in three testing sessions, each consisting of MR imaging and neuropsychological testing. TBI patients were tested at three visits, Visit 1, acquired approximately 2 months (mean= 63 days) post-injury (ranging from 28 days to 81 days), Visit 2, approximately one year (mean= 318 days) after Visit 1 (ranging from 226 days to 381 days) and Visit 3, approximately three years (mean= 1187 days) after Visit 2 (ranging from 956 days to 1651 days). Controls also participated in three visits with approximately one year (mean= 286

days) between Visit 1 and Visit 2 (ranging from 74 days to 374 days) and three years (mean= 1096 days) between Visit 2 and Visit 3 (ranging from 778 days to 2011 days).

Neuropsychological examination

On the day of each scan, a neuropsychological battery that included: COWAT (Cognitive Oral Word Association Test), WRAT-III (Wide Range Achievement Test- reading subtest, an approximation of pre-morbid intelligence), Finger Tapping (during which participants tapped a lever for 10 seconds as fast as possible for three trials and the average was used), Digit Span (a measure of working memory) and Trail Making Tests A (a visual-motor speed task) and B (a combination task requiring both visual-motor skill and rapid cognitive set shifting) was administered to each participant. These tests were selected based on previous research in our laboratory suggesting their probable relevance to TBI induced behavioral changes. Some of our analyses also included Trails B cognitive component scores which were calculated by subtracting each subject's Trails A score (visuomotor) from his or her Trails B score (visuomotor and cognitive) to isolate the cognitive component of the Trails B task. Statistical analysis of neuropsychological test results was performed as follows: For each test, a general linear model repeated measures test was carried out in SPSS 20.0. From these models, main effects of group and time as well as a group by time interaction were derived. Simple effects analyses were performed by using independent samples one-tailed t-tests to assess between groups differences in task performance at each time point, and by using paired samples one-tailed t-tests to assess within groups changes in task performance across time points.

Magnetic resonance imaging

All participants underwent magnetic resonance on a General Electric 3.0 T (Waukesha, WI) MRI system with a quadrature birdcage head coil. Structural scans included an axial T1-weighted inversion recovery-prepped spoiled gradient echo scan (inversion time = 600 ms, repetition time (TR) / echo time (TE) / flip angle = 9 ms/1.8 ms/20°; acquisition matrix = 256 × 192 interpolated to 256 × 256; field of view (FOV) = 240 mm; and 124 slices 1.2mm thick). Diffusion tensor imaging was performed using a cardiac-gated, diffusion-weighted sequence with the following parameters: 12 directions with diffusion weighting of 1114 s/mm^2 and a non-diffusion weighted reference image (B0); TR = 10–15s; TE = 78.2 ms; number of averages: 3; acquisition matrix = 120 × 120 interpolated to 256 × 256; FOV = 240 mm; 39 contiguous 3mm thick axial slices. The scan resulted in .9375 x .9375 x 3mm voxels. Prior to the diffusion-weighted scan, high order shimming was performed to minimize EPI distortions. A neuroradiologist (HR) reviewed all structural MRI images to identify the location and extent of lesions associated with the TBI and to identify non-injury related brain abnormalities that might exclude participants from the statistical analyses. Additionally, a high resolution 2D axial T2* gradient echo sequence sensitive to both DAI and contusions, was collected for evaluation by a neuroradiologist who confirmed the presence of brain injury. Imaging parameters were as follows: gradient echo read-out with TR = 325 ms, TE = 20 ms; flip angle= 15°; acquisition matrix = 256×192 interpolated to 256×256; FOV= 240 mm; 22 5mm thick axial slices, with a 1mm skip between slices; and receiver bandwidth = ±15.83 kHz.

Diffusion tensor image processing

Image distortions in the DTI data caused by eddy currents were corrected using a 2D affine co-registration function, align linear, in the Automated Image Registration software

package (http://www.loni.ucla.edu/Software/AIR). Non-linear image distortion from static field (B0) inhomogeneities was corrected using the acquired field map and implemented in the prelude (Phase Region Expanding Labeller for Unwrapping Discrete Estimates) and fugue (FMRIB's Utility for Geometrically Unwarping EPIs) tools from the FSL software suite (Smith et al., 2004). After distortion corrections, three-dimensional maps of the diffusion-tensor and derived measures, fractional anisotropy (FA), axial diffusivity (determined using the principle eigenvalue, L1) and radial diffusivity (determined using the average of the secondary and tertiary eigenvalues, L2 and L3), were calculated. For each subject, the FA, radial diffusivity, and axial diffusivity maps from Visits 2 and 3 were then co-registered to the corresponding maps from Visit 1 using flirt (FSL) 12-parameter affine co-registration. Normalization was then performed using fNIRT in FSL. Normalization of the Visit 1 maps to the FSL FMRIB58_FA_1mm template was performed for each subject. The transformation derived from this normalization was then applied to the co-registered maps acquired during Visits 2 and 3. The maps from each time point for each measure were visually checked for alignment to each other and the template.

Statistical analysis

Primary statistical analyses were performed on FA maps using factorial ANOVA statistical modules in SPM8. Gender was used as a covariate in all analyses. Because this study focused only on white matter change, all SPM analyses were restricted to regions within a white matter mask to increase our power to detect longitudinal change. The white matter mask was created by applying a threshold of .5 (Figure 1) to the mean FA map of all subjects, and then binarizing the resulting image using fslmaths (FMRIB Software Library). To determine whether there were overall group differences in longitudinal FA change, we tested for an interaction

between group and time within the factorial model. The hypothesis that there would be longitudinal change within the TBI group was tested using simple effects analyses, also within the factorial model framework.

Secondary statistical analyses using axial and radial diffusivity maps were also performed using factorial ANOVA statistical modules in SPM8. Because these analyses were employed to further examine the causes of FA change, axial and radial diffusivity analyses were restricted to regions where significant FA results were observed. We hypothesized that increases in FA would be driven by decreased radial diffusivity and/or increased axial diffusivity, and also conversely that decreases in FA would be driven by increased radial diffusivity and/or increased axial diffusivity.

Correlations with neuropsychological test scores were assessed using linear regression implemented in SPM8, where test scores were independent variables and FA maps were the dependent variables. We hypothesized that we would see positive regional correlations between FA and task performance. In addition to direct correlations between neuropsychological test scores and FA values, we also tested hypotheses concerning how changes in FA and changes in neuropsychological performance might correlate. In these cases, differences in neuropsychological test scores between two time points were calculated for each subject, as were differences in FA during the same interval. FA differences were determined by subtracting the later maps from the earlier maps for each subject. These difference correlations with changes neuropsychological test scores were also assessed using linear regression implemented in SPM8, where changes in test scores were independent variables and FA change maps were the dependent variables. All correlation analyses of neuropsychological measures were limited to regions within the white matter mask used in general FA analyses.

Statistical threshold

A voxel-level threshold of α=0.001 (uncorrected) was used for all contrasts. Multiple-comparison correction for FA analyses was performed using estimates from a Monte Carlo simulation performed with AlphaSim to achieve a corrected cluster-level threshold of α=0.05 (Forman et al., 1995). The Monte Carlo simulation determined based on randomly computed images that a cluster with 581 voxels with the same dimensions, voxel probability threshold, and smoothness parameters as FA images inputted for analysis would be unlikely (at the α=0.05 cluster-level corrected threshold) to be significant only by chance. Thus, a cluster level threshold of 581 voxels was used in all FA analyses. For secondary factorial analyses of axial and radial diffusivity and regression analyses used to test neuropsychological correlations cluster levels were determined individually for each SPM at the significance levels noted above.

Results

Demographic and behavioral results

There were no significant differences in age (t=-1.131, df=19, two-tailed p=.272) or years of education (t=-1.861, df=19, two-tailed p=.078) between the TBI and control groups. There were significantly more males in the TBI group compared to the control group ($\chi 2(1,12)=4.535$, p=.033). Demographic results are shown in Table 1. Repeated measures analyses of neuropsychological test results indicated a main effect of group in the DSPAN, Trails B and COWAT tasks, as well as a group by time interaction for the COWAT task (Table 2). Simple effects analyses of neuropsychological tests performance indicated that TBI patients' performance was significantly worse than that of controls on Digit Span, Trails A and Trails B at Visits 1, 2 and 3. TBI patients also differed from controls on the COWAT at Visits 1 and 2. No significant group differences were seen in WRAT or Finger Tapping. TBI patients demonstrated significant improvements on the DSPAN, Trails A, Trails B and COWAT tests between Visit 1 and Visit 2, and also showed significant improvement on the Trails A, Trails B and COWAT tests between Visit 1 and Visit 3. Controls demonstrated significant improvement on the Trails A and Trails B tasks between Visit 1 and Visit 2. All neurological test results are shown in Table 2.

DTI results

The FA factorial analysis revealed a group by time interaction in the genu of the corpus callosum (Figure 2). Analyses of the secondary metrics (using separate factorial models for axial diffusivity and radial diffusivity maps) demonstrated that there was also a group by time interaction in this region in the radial diffusivity model, but not the axial diffusivity model

(Figure 2). Together these analyses indicate that the group by time interaction observed in the genu in the FA analysis was driven by changes in radial (not axial) diffusivity.

A main effect of time was observed throughout the corpus callosum in the primary FA analysis (Figure 3). A corresponding main effect of time was observed in this region in the radial, but not the axial, diffusivity analysis (Figure 3). A main effect of group was observed in white matter tracts throughout the brain, including the cerebral peduncle, inferior and superior longitudinal fascicule (ILF and SLF), internal and external capsule, inferior fronto-occipital fasciculus, sagittal stratum, corpus callosum, fornix, optic radiations, thalamic radiations, uncinate fasciculus and corona radiata (Figure 4). Axial and radial diffusivity analyses revealed a main effect of group in genu, fornix and ILF in the axial model and a main effect of group throughout the corpus callosum, as well as in the fornix, ILF, optic radiations and thalamic radiations in the radial model (Figure 4).

Simple effects analyses within the FA factorial model demonstrated that TBI subjects exhibited a significant decrease in FA throughout the corpus callosum between the first and third visits (Figure 5a). We hypothesized that this change would be driven either by a decrease in axial diffusivity, an increase in radial diffusivity, or some combination of the two. Analyses of the secondary metrics limited to this corpus callosum region were used to test this hypothesis. Results showed that TBI subjects did not demonstrate any decreases in axial diffusivity in this region, while they did exhibit significant increases in radial diffusivity in both the genu and isthmus of the corpus callosum (Figure 5a). Controls did not demonstrate significant longitudinal FA decreases in any brain regions.

In order to determine whether the decrease in FA in the corpus callosum within the TBI group between the first and third visits was driven primarily by early changes during the first

interval or late changes during the second interval, each interval was assessed individually within the FA factorial model. These secondary analyses were limited to the corpus callosum region where a change had been observed between the first and third time points. Results demonstrated that significant changes could be seen in the genu and body of the corpus callosum during the first interval (Figure 5b), but no significant clusters were identified during the second interval. Axial and radial diffusivity analyses within this region indicated that an increase in radial diffusivity was present in the genu during the first interval among TBI subjects (Figure 5b), however no accompanying decrease in axial diffusivity was observed.

Simple effects analyses within the FA factorial model also demonstrated that TBI subjects exhibited significant FA increases in bilateral regions of the superior longitudinal fasciculus (SLF) as well as in an optic radiation region on the left side of the brain between the first and third visits (Figure 6). Again, we hypothesized that changes observed among TBI patients would be driven either by decreases in axial diffusivity, increases in radial diffusivity, or some combination of the two. Analyses of the secondary metrics limited to these regions were used to test this hypothesis. Results showed that TBI subjects did not demonstrate any increases in axial diffusivity in these regions between the first and third visits, however significant decreases in radial diffusivity were observed in all regions tested (Figure 6). Controls did not exhibit significant longitudinal FA increases in any brain regions.

In order to determine whether the increases in FA in the bilateral SLF and left optic radiation within the TBI group between the first and third visits were driven primarily by early changes during the first interval or late changes during the second interval, each interval was assessed individually within the FA factorial model. These secondary FA increase analyses were limited to the regions where a change had been observed between the first and third time points.

No significant clusters were identified during either the first or second intervals individually, suggesting that the observed change occurred gradually over the four-year study duration.

Between groups simple effects analyses demonstrated that the TBI group had reduced FA compared to the control group in the cerebral peduncle, ILF, SLF, internal and external capsule, inferior fronto-occipital fasciculus, sagittal stratum, corpus callosum, fornix, optic radiations, thalamic radiations, uncinate fasciculus and corona radiata at all three visits. There were no regions in which the TBI group had greater FA than the control group at any of the three time points. We hypothesized that FA reductions would be driven by a combination of reduced axial and increased radial diffusivity. Secondary analyses confirmed this hypothesis, demonstrating that the TBI group had reduced axial diffusivity compared to controls in parts of the cerebral peduncle, external capsule, internal capsule, optic radiation, fornix, SLF and ILF at all three time points. Increased radial diffusivity among TBI patients was observed in all regions in which group differences in FA were observed at all three time points.

Neuropsychological correlations

Contrary to our hypothesis, we did not observe any correlations between an individual's GCS score and regional white matter FA at any of the three time points. Of the seven neuropsychological measures tested for correlation with FA, two tests, Trails A and dominant hand finger tapping, were significantly correlated with regional FA values among the TBI subjects. No significant correlations were observed between FA and neuropsychological test performance among control subjects. Finger tapping scores correlated positively with FA in the splenium of the corpus callosum at the second time point (Figure 7). A lowered statistical threshold α=.01 enabled observation of smaller splenium clusters when the equivalent

correlational tests were run for the first and third time points, however only the second time point result surpassed significance levels employed in this study. Trails A performance correlated positively with FA in bilateral regions of the sagittal stratum at the first time point, and a unilateral region in the left sagittal stratum at the second time point (Figure 8a).

Correlations between FA changes and changes in neuropsychological measures over time were also significant for two neuropsychological measures, Trails A and the Trails B cognitive component. No equivalent correlations were observed among controls for any measure. There was a positive correlation between change in the Trails A score between the first and third time points and change in FA over the same duration (where an increase in FA was associated with a reduction in time to complete Trails A) in the right sagittal stratum (Figure 8b). No significant correlations were observed between change in Trails A score and change in FA during either sub-interval of the study (from time one to time two or from time two to time three). A positive correlation between FA change and change in the Trails B cognitive component between time one and time three was observed in the left superior SLF and the right optic radiation (Figure 9). Analysis of this correlation during each of the sub-intervals did not reveal any regions of significant clusters between the first and second time point, but a significant cluster was observed in the right posterior SLF between the second and third time points (Figure 9).

Discussion

Longitudinal brain changes following TBI are sparsely documented. In this study we examined TBI patients over a period of 4 years and found that rather than showing a circumscribed period of brain degeneration following injury, TBI involves a protracted period of brain change that continues for several years. The results of this study suggest that studying alterations in brain white matter may provide clues to neuropsychological function following TBI, and potentially inform upon the clinical course of patients following injury.

In our study, we found significant effects in the corpus callosum, which is commonly injured in TBI. The group by time interaction observed in the FA factorial model combined with the simple effects analyses indicates that the TBI subjects demonstrated a significantly greater reduction in FA in the genu of the corpus callosum during the first year post-injury than during the subsequent three years of the total follow-up period. This result is commensurate with previous work on a different subset of individuals in this cohort indicating FA reductions in this region during the first year post injury (Bendlin et al., 2008), as well as other previous studies that have also found longitudinal white matter decline in this region during the first year (Wu et al., 2010; Xu et al., 2007). Our results also demonstrated that the observed FA effect was driven by increased radial, rather than decreased axial, diffusivity, which is consistent with previous observations (Kumar et al., 2010; Sidaros et al., 2008). Other recent research has indicated that initial injury to the genu is highly predictive of patient outcome (in terms of general disability), however we did not observe correlations between FA change in this region over time and change in neuropsychological task performance (Matsukawa et al., 2011).

The main effect of time combined with simple effects analyses indicate that TBI patients experienced a continued decline in FA throughout the corpus callosum that continued throughout

the duration of the four year period studied, and that this decrease was driven by increases in radial diffusivity. It has been suggested that initial tearing, shearing, and misalignment of axons initiates an inflammatory cascade that leads to further white matter damage, myelin loss, and gliosis, and we expect that these processes were critical to the gradual, long-term FA reductions observed here (Povlishock, 2000).

The main effect of group combined with between-groups simple effects analyses indicate that TBI subjects exhibited reduced FA in several major tracts, that this difference was persistent for at least four years post-injury, and that a combination of reduced axial and increased radial diffusivity gave rise to these FA differences. These results collectively demonstrate that in several major tracts there is reduced directional coherence of white matter among our TBI subjects that is both widespread and long-lasting. These between-groups differences are consistent with cross-sectional observations (Bendlin et al., 2008; Chan et al., 2003; Kiraly & Kiraly, 2007; Nakayama et al., 2006; Sidaros et al., 2008; Wang et al., 2011; Xu et al., 2007).

Our simple effects results also demonstrate that TBI subjects show increases in FA in the SLF bilaterally as well as in a portion of the optic radiation during the course of the study, potentially signifying improvement of white matter integrity or alternatively showing loss of crossing fibers in these brain regions. No previous study that we are aware of has demonstrated longitudinal increases in FA among TBI patients. This is likely because no previous study that we are aware of followed patients for four years as the current study did, and the FA increases we observed appear to have taken place gradually during the four year study duration. Previous research has, however, identified apparent improvements in FA in animal models of TBI (Rubovitch et al.), and in either axial or radial diffusivity among human TBI patients (Kumar et al., 2010; Sidaros et al., 2008), indicating that our present finding is potentially replicable. This

evidence of subtle neurological recovery merits further investigation, particularly in clinical settings. The secondary result indicating that this increase in FA was driven by a longitudinal decrease in radial diffusivity (rather than an increase in axial diffusivity) could suggest improved myelin integrity within the tract, however it is equally plausible that progressive loss of damaged axons that is observed in animal models of TBI (Creed, DiLeonardi, Fox, Tessler, & Raghupathi, 2011) may underlie the observed FA changes in our TBI cohort.

The fact that longitudinal changes in radial (rather than axial) diffusivity were found in the same regions where FA changes were found warrants further attention. In the literature, findings concerning radial diffusivity and TBI tend to be relatively consistent across studies, whereas findings relating to axial diffusivity are inconsistent. Many studies have reported longitudinal increases in radial diffusivity in the absence of changes in axial diffusivity, and like in the current study these changes have frequently been localized to the corpus callosum (Ewing-Cobbs et al., 2008; Mac Donald, Dikranian, Bayly, Holtzman, & Brody, 2007; Newcombe et al., 2007; Tasker, Westland, White, & Williams, 2010). Differences in axial diffusivity, however, have been inconsistent, with some groups finding increases (Sidaros et al., 2008; Tasker et al., 2010), decreases (Li, Li, Feng, & Gu, 2011) or no change (Mac Donald et al., 2007) in both the corpus callosum and other regions. These inconsistencies may be due to differences in how long after injury patient scans were obtained. While FA variations may be related to myelin change, axonal change, or differences in directional coherence of fibers (e.g. presence of absence of crossing fibers), changes in axial diffusivity are thought to be associated primarily with axonal changes while changes in radial diffusivity are thought to relate to myelin changes (Alexander et al., 2007; Song et al., 2002; Xie et al., 2010). These findings indicate that progressive FA loss observed among our subjects was likely driven by progressive myelin pathology, possibly due to

persistent inflammation (Ramlackhansingh et al., 2011). While progressive FA increases observed could have been driven by improvements in myelin integrity, the removal of axons with damaged myelin by phagocytotic processes likely also contributed to our result.

While it is not plausible to determine why some regions showed FA increases while others showed decreases with imaging data alone, it is nonetheless instructive to speculate on this matter. One likely contributing factor is the specific injuries sustained by this cohort of TBI patients. The corpus callosum is frequently that center of severe damage during TBI, and the subjects in this cohort were no exception. Several subjects' initial radiology notes included mentions of corpus callosal damage, but none mentioned other tracts specifically. It is possible, therefore, the continued deterioration was observed in regions with greater initial damage. Another complementary possibility is that differential tract properties promote different responses to insult. Research with animal models has demonstrated that astrocytic proliferation can occur in responses to focal injury, but only certain subclasses of neurons are capable of this type of structural remodeling (Blizzard et al., 2011). Further research, most likely in animal models, is necessary to determine why certain regions continue to deteriorate post-injury while others remain static or even improve.

GCS score and white matter integrity were not correlated in TBI patients, likely due to the heterogeneity of patients' injuries. It is probable that more severely injured individuals had greater white matter damage near impact sites, but variable injury locations across subjects precluded group-wise identification of these differences. The neuropsychological correlations we observed were consistent with our hypothesis that FA and neuropsychological task performance would correlate positively. In TBI patients, damage to the splenium indexed by lower FA was associated with slower finger tapping speed, consistent with previous studies linking corpus

callosum to manual motor tasks (Caeyenberghs, Leemans, Coxon et al., 2011; Caeyenberghs, Leemans, Geurts et al., 2011; Muetzel et al., 2008).

Faster performance on the Trails A test was associated with higher FA in the sagittal stratum, an interesting finding given that this tract is known to be implicated in visuomotor functions. Individuals with greater damage to this tract likely had more difficulty completing the task (Hao et al., 2011; Makris et al., 2005). Over time, this relationship held, with a subset of TBI patients showing longitudinal improvements in Trails A completion speed between the first and third time points and a corresponding increase in FA in the sagittal stratum over the same interval.

Correlations were also observed between change in the Trails B cognitive component score over time and change in FA values among TBI patients. A correlation was observed between the first and third time points in the left SLF and the right optic radiation, as well as in the right SLF between the second and third time points. Previous work has demonstrated that FA in the SLF is is associated with complex functions such as attentiveness, working memory and reading skills (Frye et al., 2010; Karlsgodt et al., 2008). The SLF subserves a wide variety of connective functions, and the longitudinal improvements in directional coherence of fibers within this tract among TBI patients in this study may have contributed to improved scores on the Trails B cognitive component measure. Interestingly, we also found a relationship in the optic radiation (a tract relevant to the relay of visual information from the lateral geniculate nucleus to the visual cortex); this may suggest that subtracting Trails A scores from Trails B scores does not entirely remove the visuomotor element of the task, or possibly suggesting this tract is important for subserving the cognitive component of a visuomotor task.. Overall, our neuropsychological correlations demonstrate that the differences in FA observed in our study do

indeed have an impact on cognitive and motor function, and that subtle increases in FA over time reflect white matter change that is related to improved functionality in patients. While it should be noted that the neuropsychological correlations reported in the current study may be specific to the set of patients included and their particular patterns of white matter damage, it is nonetheless informative to identify functional relevancies of white matter change.

In addition to the primary analyses and results presented in this paper, we also conducted an investigatory simple effects analysis of whole-brain radial diffusivity changes within our patients. This analysis was carried out within the radial diffusivity factorial model used in our primary analysis, but the search was not restricted to regions demonstrating FA change. The results of this analysis, which used the same statistical parameters as our primary FA analysis, indicated that within our cohort longitudinal decreases in radial diffusivity (approximating improvements in myelin integrity) were found in regions throughout the brain during the four year study duration. Regions included superior and inferior longitudinal fasiculi, internal and external capsules, the descending corticospinal tract, and forceps major and minor (Supplementary Figure). While this analysis is beyond the planned scope and goals of the current study, it is provocative and may be informative to future research.

The results of this study likely have significant clinical relevance. Specifically, it is notable that we observed white matter changes occur for several years post-injury because the continued malleability of the injured brain holds promise for the effectiveness of treatments well beyond the three to six month window in which treatments are typically prescribed. The FA increase observed in this study has particular relevance to treatment options, as it reflects plasticity and represents a potential physiological basis of rehabilitation. In order to truly assess the relevance of these results to clinical applications, a clinical trial study would be necessary. In

such a study, DTI would be used as an outcome measure with the expectation that patients undergoing treatment would exhibit less deterioration or greater improvement in regional brain white matter integrity. A positive result would underscore the clinical relevance of our findings.

The current study has methodological limitations that should be considered. Firstly, the results of our study may be limited by methodological limitations imposed by performing a patient and control comparison, for example, it is possible that preprocessing of imaging data and even MR signal of interest can be affected differentially by group. Voxel-wise comparisons of brain images is dependent upon accurate alignment to a template; in order to minimize error, all FA maps were visually inspected for within-subject tract alignment to the template and alignment across all participants. Another potential limitation concerns the greater ratio of women to men in the control group compared to the TBI group. Due to this, we used gender as a covariate in all analyses. In this study we were not able to account for the intensity or duration of rehabilitations programs in which some of our patients participated. Future clinical trials are critical to understanding how rehabilitation programs impact neurological recovery.

Concurrent volume loss exhibited by TBI patients could also have confounded our results. Volume loss following injury is well established in the TBI literature (Levine et al., 2008; Merkley et al., 2008; Sidaros et al., 2009), and has also been observed in this particular cohort of patients (Bendlin et al., 2008). While any DTI study done on this patient population will have results obtained in the context of volume loss, it is nonetheless important to acknowledge that tissue contraction, in addition to microstructural reorganization, may contribute to observed changes in DTI metrics. To ensure that our results were not due solely to volumetric changes, we conducted a native space region of interest analysis on the genu of the corpus callosum. The genu was selected because it is a functionally relevant region and is easily

identifiable. FA values were extracted from 3mm spherical ROIs placed in the center of the genu in each participant's native space DTI images from Visits 1, 2 and 3. Independent-samples two-tailed t-tests showed that TBI patients had reduced FA compared to controls at all three time points (Visit 1: Controls- m=.77, TBIs- m=.67, p=.005; Visit 2: Controls- m=.79, TBIs- m=.63, p=.0003; Visit 3: Controls- m=.79, TBIs- m=.61, p=.001). Paired-samples two-tailed t-tests showed that TBI patients demonstrated a reduction in FA between Visits 1 and 2 (p=.01) as well as between visits 1 and 3 (p=.04). Paired samples t-tests showed no longitudinal changes within the control group. These results mirror the observations made in our whole-brain analysis, and partially allay concerns about the confounding effects of concurrent volume change.

Finally, the results of this study may be limited by the small number of participants that were followed through all three time points. The TBI population and college aged controls are both itinerant populations, and therefore difficult to track for long periods of time. Notwithstanding this limitation, follow-up of TBI patients over three time points makes this an extremely valuable sample. Furthermore, we did not find significant differences in age, education or injury severity within either group between those who dropped out of the study and those who completed all three visits, suggesting that selective drop-out did not bias the results.

In this study, we show that TBI patients exhibit longitudinal white matter changes that continue for at least four years post injury. These changes include both progressive reductions in FA (observed in the corpus callosum) as well as progressive FA increases (observed in the bilateral SLF). Within the regions where FA changes were found, radial diffusivity alterations were present—suggesting myelin changes continued to occur throughout the period studied. Furthermore, neuropsychological correlations indicate that diffusion metrics are related to cognitive and motor abilities, and that improved directional coherence within tracts is relevant to

improved task performance in certain brain regions among TBI patients. The fact that changes continued to develop for several years post-injury provides support for the hypothesis that TBI is not merely an event but rather the initiation of a prolonged disease state with potentially lifelong systemic impacts. FA increases are of particular note due to the unprecedented nature of these findings. TBI is generally conceptualized as strictly a degenerative condition, and evidence of what might be long term neurological improvement could be of significant clinical importance. We strive to continue to improve our understanding of these long term effects following traumatic injury. An important area of work going forward will be to capitalize on signs of gradual long-term neurological and functional improvement observed in TBI, and to further understand the brain correlates of ameliorative change, which likely occur alongside progressive degeneration.

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Table 1: *Individual level subject demographics for patients and controls and select patient injury characteristics*

Patients

Subject	Age	Education	Sex	GCSadmit	GCS24	Hrs15	Injury Notes
1	19	12	M	3T	7	334	DAI
2	49	12	M	15	15	0	DAI, subarachnoid hemorrhage, CC damage
3	19	14	F	3T	11	91	Subarachnoid hemorrhage, subdural hematoma
4	24	14	M	3	7	662	DAI, contusions, epidural hematoma, subarachnoid hemorrhage
5	39	10	M	3T	6	835	Extensive contusions, subdural hematoma
6	45	12	M	3T	14	270	Skull fracture, frontal contusion, CC damage
7	29	13	M	3	14	179	DAI, contusions, skull fracture
8	52	16	F	11	13	97	Skull fracture, subarachnoid hemorrhage, subdural hematoma,
9	51	12	M	7	7	726	Depressed skull fracture, subdural and epidural hematomas
10	19	13	M	3T	5	49	Extensive hemorrhages, CC shearing
11	37	14	M	9	7	116	DAI, subarachnoid hemorrhage, subdural hematoma, shearing
Mean (SD)	35.0 (12.8)	13.2 (1.5)	83% M	5.7 (4.2)	9.6 (3.8)	305.4 (297.8)	

Controls

Subject	Age	Education	Sex
12	22	19	F
13	22	**15**	M
14	29	13	F
15	27	16	F
16	25	12	F
17	19	16	F
18	36	16	M
19	24	13	M
20	51	13	M
Mean (SD)	29.2 (9.7)	14.8 (2.2)	44% M
p	.272	.078	.033

Note: Age of participants indicates the mean age of participants at the start of the study. P-values are based on the results of two-tailed independent-samples t-tests for age and education and a chi-squares test for gender proportions. Abbreviations are as follows: GCSadmit= Glasgow Coma Scale score at hospital admission, GCS24= Glasgow Coma Scale score 24 hours post-injury, Hrs15= Number of hours before patient reached a GCS score of 15, DAI= Diffuse axonal injury, CC= corpus callosum, T= Patient was intubated at the time of GCS assessment.

Table 2
Neuropsychological test results

Task	Time 1 n, **Mean** (SD)	Time 2 n, **Mean** (SD)	Time 3 n, **Mean** (SD)
	TBI Group Results		
WRAT-III	12, **44.7** (6.5)	12, **44.5** (12.3)	11, **45.6** (5.7)
DSPAN[1]	12, **14.3*****[†] (3.6)	12, **15.6*** (3.7)	11, **15.6*** (4.6)
Trails A	12, **42.0***[†] (21.5)	12, **30.8*** (6.4)	12, **28.7*****[†] (7.1)
Trails B[1]	11, **101.3***[†] (29.0)	12, **78.6*** (20.9)	12, **68.8***[†] (36.4)
COWAT [3]	12, **21.2***[†††] (10.2)	12, **33.8*** (10.6)	12, **39.6**[††] (12.4)
FT	10, **44.1** (14.7)	12, **45.9** (7.6)	12, **45.7** (6.9
	Control Group Results		
WRAT-III	8, **50.0** (5.1)	7, **50.0** (6.1)	9, **55.7** (5.2)
DSPAN	8, **20.1** (3.4)	7, **20.6** (3.9)	9, **20.4** (5.2)
Trails A	8, **25.1** (6.9)	7, **24.6**[††] (5.4)	9, **20.3** (4.5)
Trails B	8, **55.0** (25.6)	7, **51.5**[†] (20.4)	9, **38.6** (7.1)
COWAT	8, **41.8** (7.8)	7, **44.6** (8.9)	9, **45.9** (11.9)
FT	6, **45.6** (10.8)	7, **48.9** (6.9)	9, **48.3** (6.8)

Note: Test abbreviations are as follows: WRAT-III= Wide Range Achievement Test (reading subtest), DSPAN= Digit Span Test, Trails A= Trail Making Test A (motor), Trails B= Trail Making Test B (motor and cognitive), COWAT= Cognitive Oral Word Association Test, FT DOM= Dominant Hand Finger Tapping Test. Means are of raw scores. Repeated measures analyses based on general linear models were carried out for each neuropsychological test and significant results ($p<.05$) are noted next to task names in the following manner: [1] indicates a main effect of group, [2] indicates a main effect of time and [3] indicates a group by time interaction. Between-groups differences were calculated using independent samples one-tailed t-tests, and significance levels are denoted as follows: are *$p<.05$, **$p<.01$, ***$p<.001$. Between groups differences are noted by the patient average scores only. Within-groups differences were calculated using paired samples one-tailed t-tests, and significance levels are denoted as follows: .[†]$p<.05$, [††]$p<.01$, [†††]$p<.001$. Differences between time 1 and 2 are noted by the first time point average, differences between time 2 and 3 are noted by the second time point average, and differences between time 1 and 3 are noted by the third time point average.

Figures

Figure 1: Because this study focused only on white matter change, all SPM analyses were restricted to regions within a white matter mask to increase our power to detect longitudinal change. The white matter mask was created by applying a threshold of .5 to the mean FA map of all subjects, and then binarizing the resulting image. This figure shows the white matter mask in three orthogonal directions. The left side of the mask is patient left and the right is patient right.

Figure 1

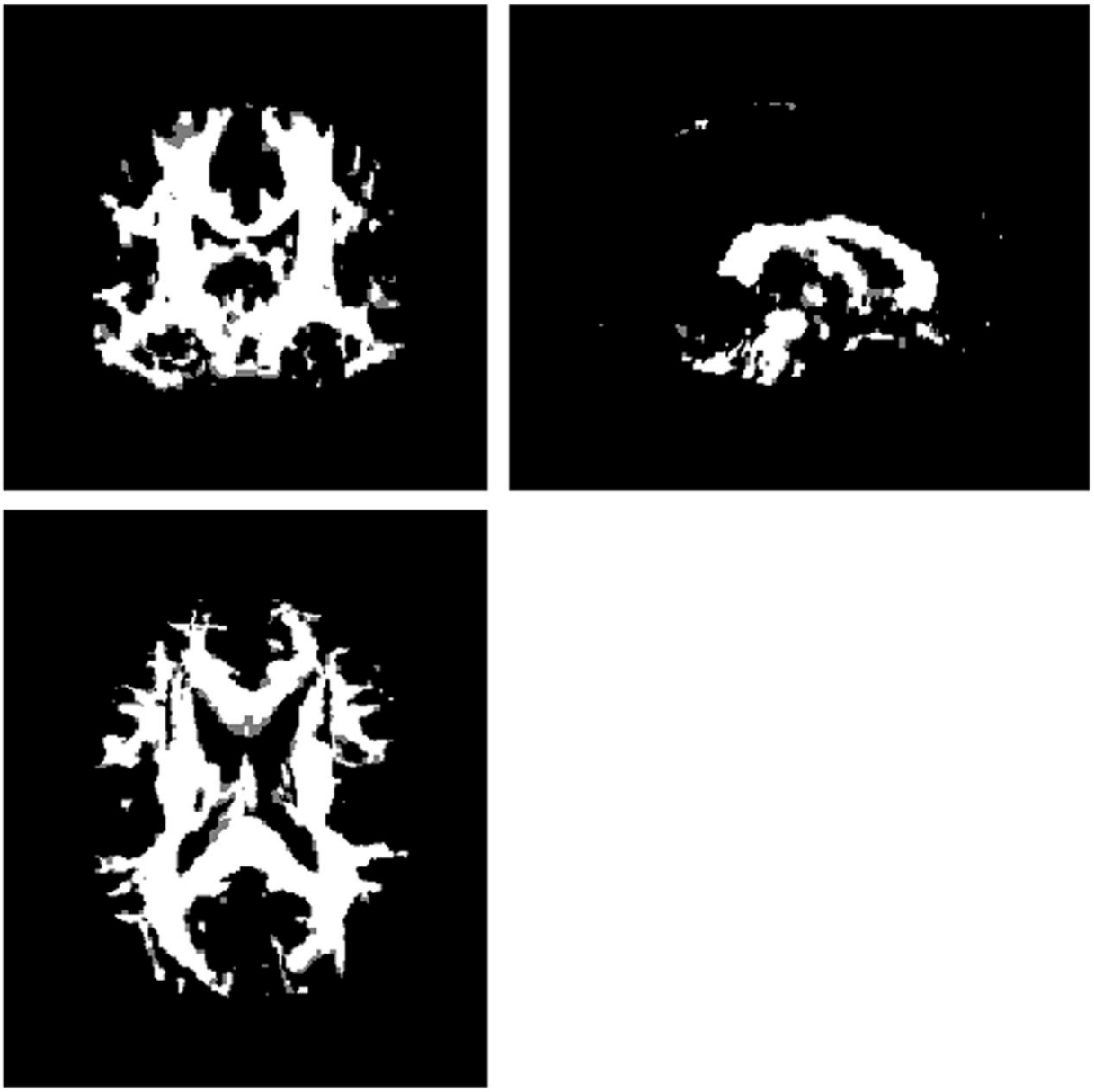


Figure 2: (Top) A significant interaction between group and interval was observed within the FA factorial model. There were inter-interval dissimilarities in group-wise patterns of FA change in the genu of the corpus callosum (red). Axial and radial diffusivity analyses were restricted to regions demonstrating an FA effect. Within this region, a significant interaction was also observed in the radial diffusivity factorial model (overlapping blue region), but not the axial diffusivity factorial model. (Bottom) This graph shows the average FA value for each subject at each of the three time points within the corpus callosum cluster demonstrating an FA effect. Controls are marked as green diamonds, TBI patients with a 24 Hour GCS score of 7 or lower are marked as pink squares, and patients with a 24 Hour GCS score of 10 or higher are marked as orange triangles. Box plots indicate the 75^{th} percentile, median, and 25^{th} percentile FA values of each group at each time point, and whiskers indicate 1.5 times inter-quartile ranges. The left side of the statistical map is patient left and right is patient right.

Figure 2

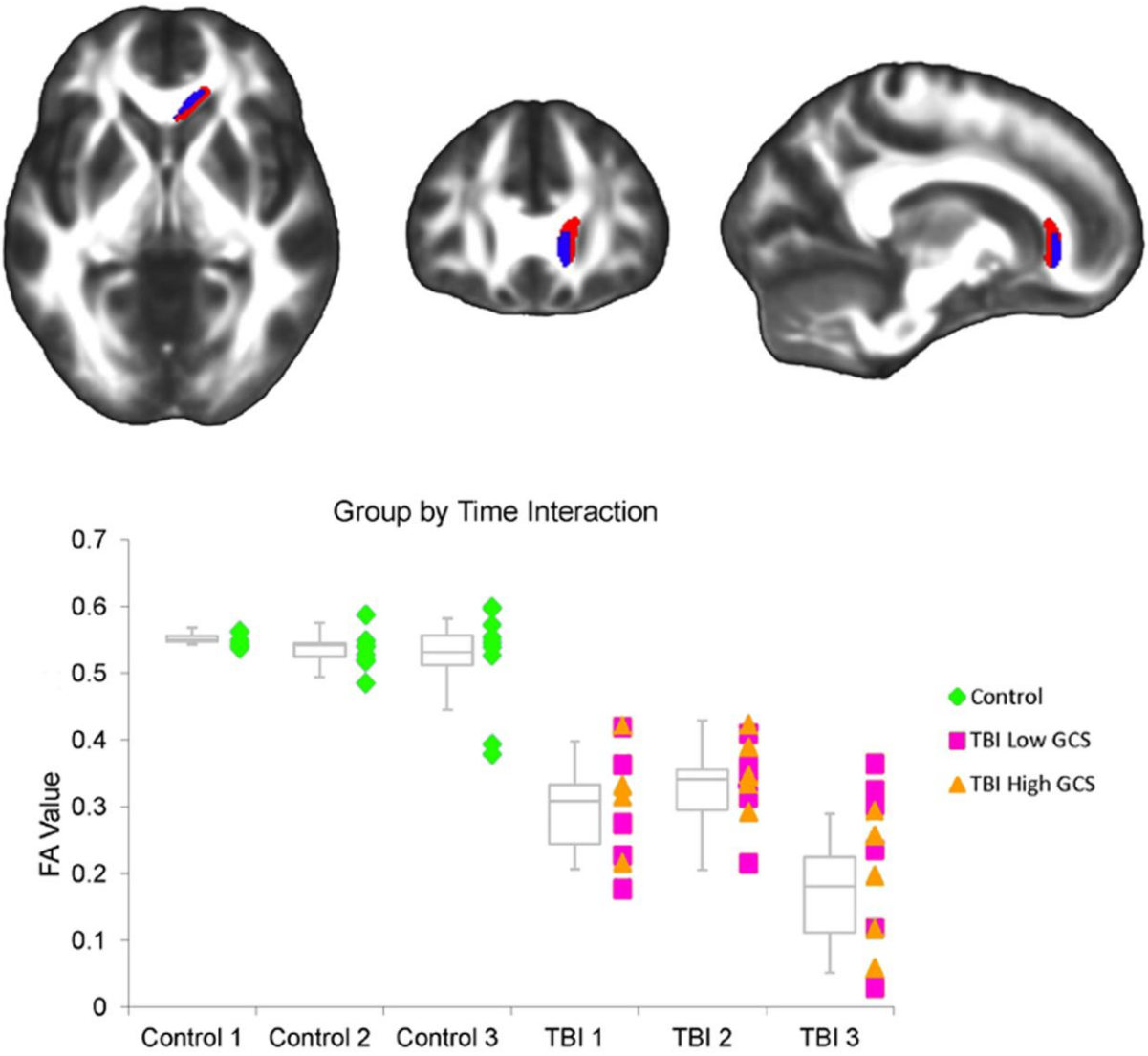

Group by Time Interaction
FA Value
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 1
Control 2
Control 3
TBI 1
TBI 2
TBI 3
Control
TBI Low GCS
TBI High GCS

Figure 3: (Top) A significant effect of time was observed throughout the corpus callosum as well as in the left SLF within the FA factorial model (red). Axial and radial diffusivity analyses were restricted to regions demonstrating an FA effect. Within this region, a significant effect of time was also observed within the radial diffusivity factorial model (overlapping blue region), but not the axial diffusivity factorial model. (Bottom) This graph shows the average FA value for each subject at each of the three time points within the corpus callosum cluster demonstrating an FA effect. Controls are marked as green diamonds, TBI patients with a 24 Hour GCS score of 7 or lower are marked as pink squares, and patients with a 24 Hour GCS score of 10 or higher are marked as orange triangles. Box plots indicate the 75^{th} percentile, median, and 25^{th} percentile FA values of each group at each time point, and whiskers indicate 1.5 times inter-quartile ranges. The left side of the statistical map is patient left and right is patient right.

Figure 3

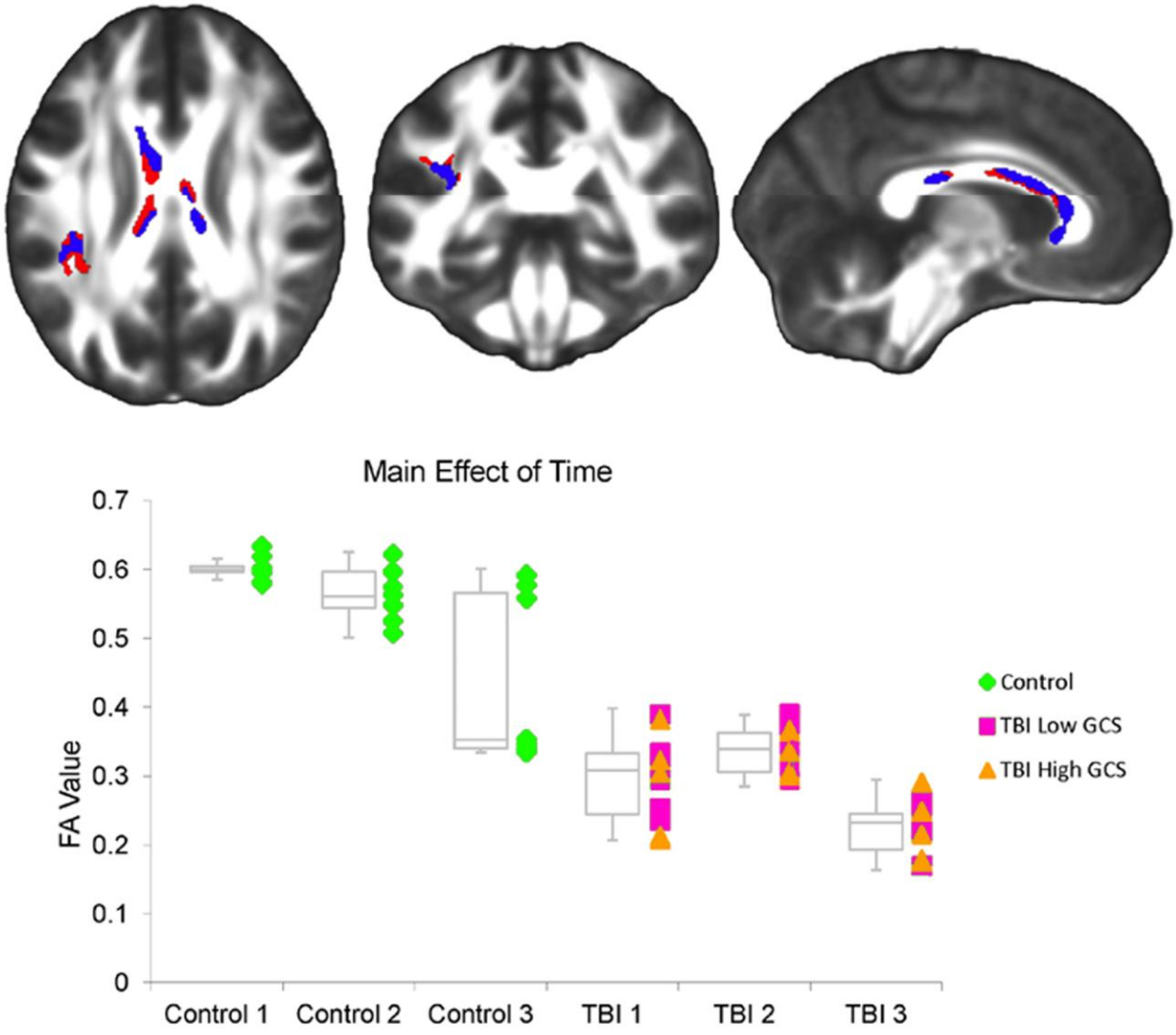

Main Effect of Time
FA Value
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 1
Control 2
Control 3
TBI 1
TBI 2
TBI 3
Control
TBI Low GCS
TBI High GCS

Figure 4: (Top) A significant effect of group was observed within the FA factorial model in several white matter tracts throughout the brain, including the cerebral peduncle, inferior and superior longitudinal fascicule (ILF and SLF), internal and external capsule, inferior fronto-occipital fasciculus, sagittal stratum, corpus callosum, fornix, optic radiations, thalamic radiations, uncinate fasciculus and corona radiata (red). Axial and radial diffusivity analyses were restricted to regions demonstrating an FA effect. Within this set of regions, a significant effect of group was also observed within the radial diffusivity factorial model (overlapping blue regions), as well as the axial diffusivity factorial model (overlapping green yellow regions). Regions in which a significant effect was seen in the FA, radial diffusivity and axial diffusivity models are shown in green. (Bottom) This graph shows the average FA value for each subject at each of the three time points within the regions demonstrating an FA effect. Controls are marked as green diamonds, TBI patients with a 24 Hour GCS score of 7 or lower are marked as pink squares, and patients with a 24 Hour GCS score of 10 or higher are marked as orange triangles. Box plots indicate the 75^{th} percentile, median, and 25^{th} percentile FA values of each group at each time point, and whiskers indicate 1.5 times inter-quartile ranges. The left side of the statistical map is patient left and right is patient right.

Figure 4

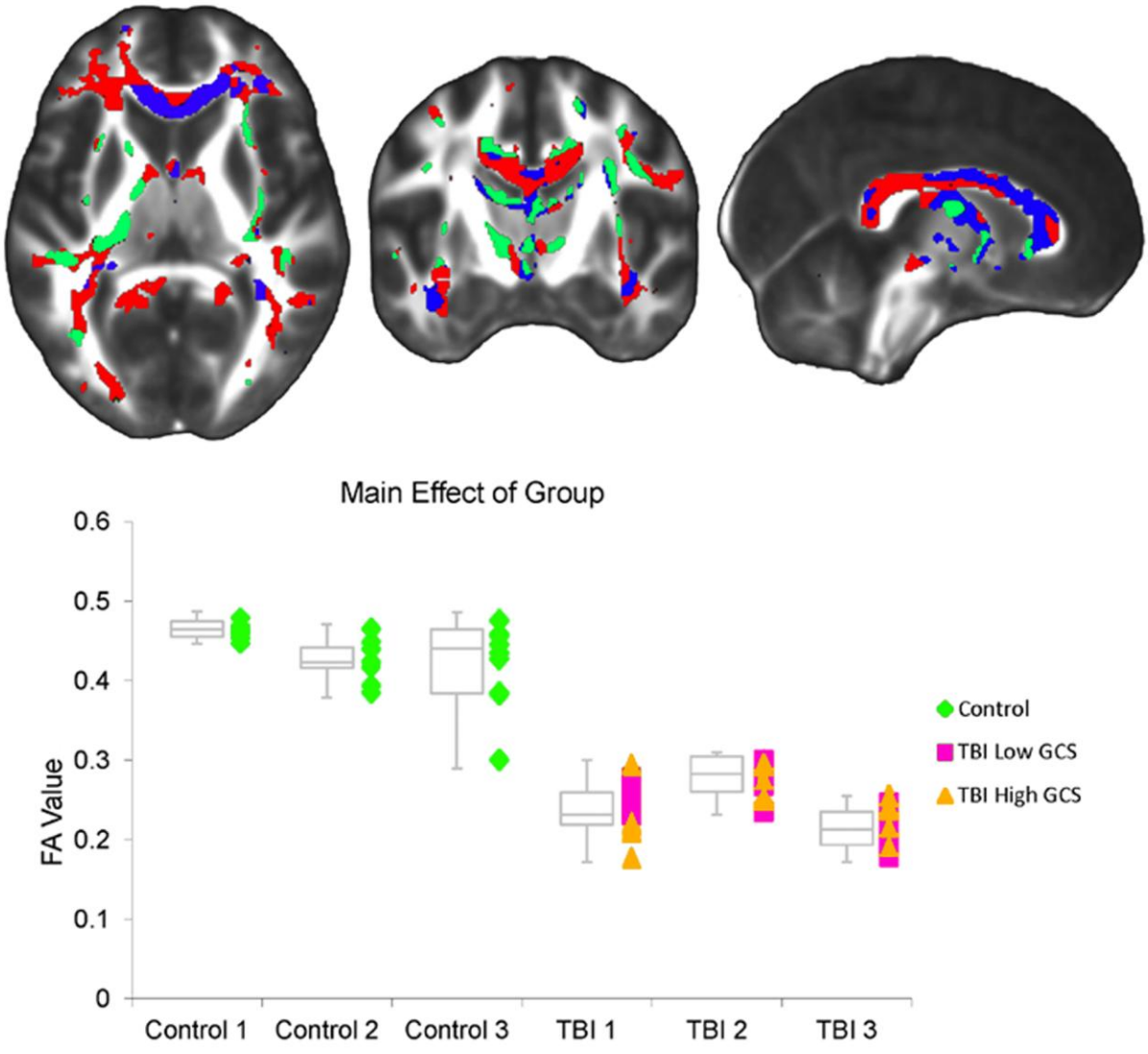

Main Effect of Group
FA Value
0.6
0.5
0.4
0.3
0.2
0.1
0
Control 1
Control 2
Control 3
TBI 1
TBI 2
TBI 3
Control
TBI Low GCS
TBI High GCS

Figure 5: (a) Simple effects analysis within the FA factorial model revealed a significant region of the corpus callosum in which TBI subjects exhibited greater FA at the first time point compared to the third time point (red), indicating a longitudinal FA reduction during the four year study duration. The equivalent test in controls did not produce any significant clusters. Secondary axial and radial diffusivity analyses were renstricted to regions demonstrating an FA effect. Axial and radial diffusivity analyses demonstrated that in this region there was a significant increase in radial diffusivity, but no decrease in axial diffusivity, during the four year study (overlapping blue region). (b) Analysis of this region during the two sub-intervals revealed that there was a significant FA reduction between the first and second time points in the genu of the corpus callosum among TBI subjects (red), but no clusters between the second and third time points. Secondary axial and radial diffusivity analyses were constricted to regions demonstrating an FA effect. Axial and radial diffusivity analyses demonstrated that in this region there was a significant increase in radial diffusivity, but no decrease in axial diffusivity, between the first and second time points (overlapping blue region). The left side of the statistical map is patient left and right is patient right.

Figure 5

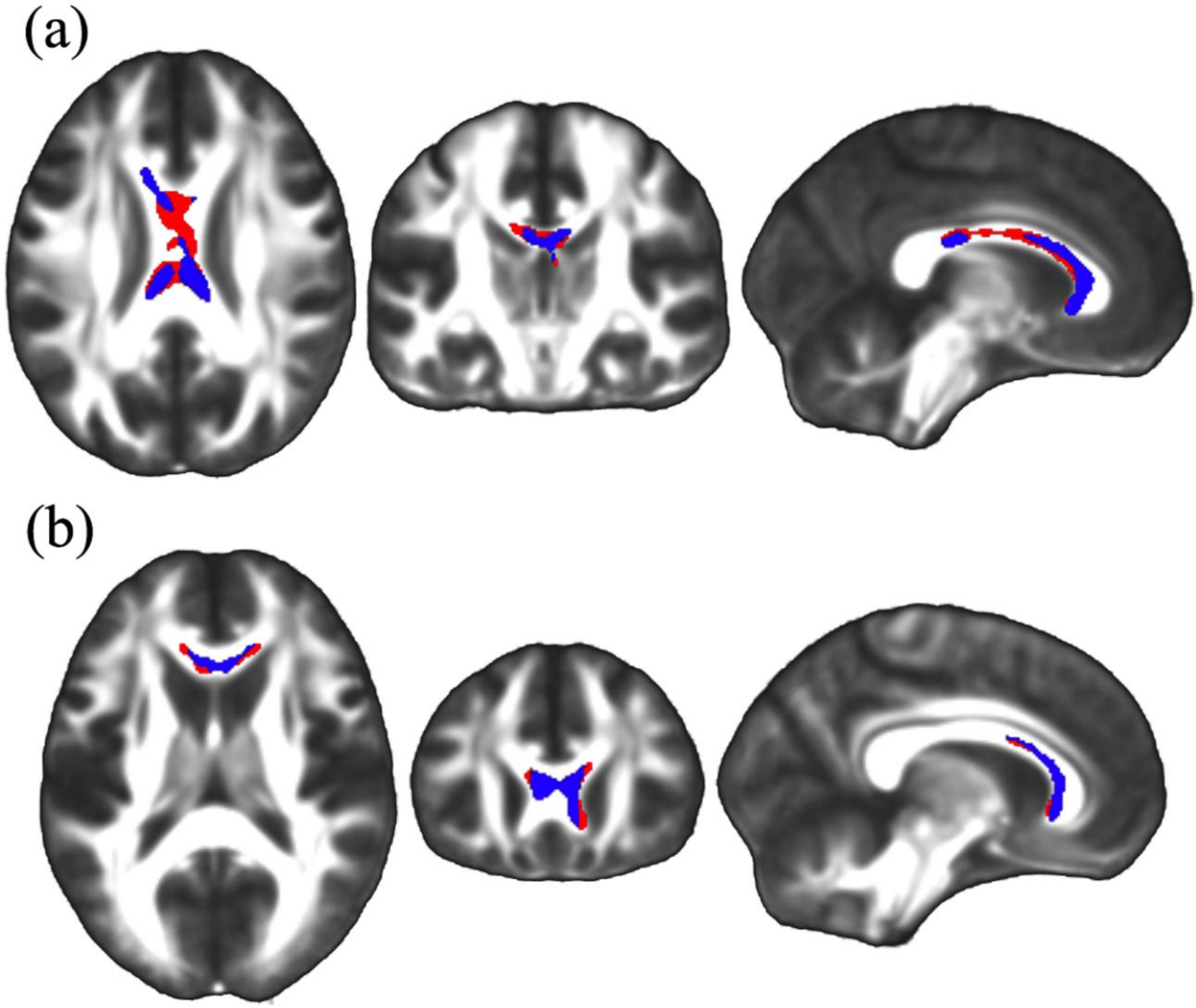


Figure 6: Simple effects analysis within the FA factorial model revealed significant regions in the bilateral SLF in which TBI subjects exhibited greater FA at the third time point compared to the first time point (red), indicating a longitudinal FA increase during the four year study duration. The equivalent test in controls did not produce any significant clusters. Secondary axial and radial diffusivity analyses were restricted to this region demonstrating an FA effect. Axial and radial diffusivity analyses demonstrated that in this region there was a significant decrease in radial diffusivity during the four year study period (overlapping blue region), but no accompanying increase in axial diffusivity. The left side of the statistical map is patient left and right is patient right.

Figure 6

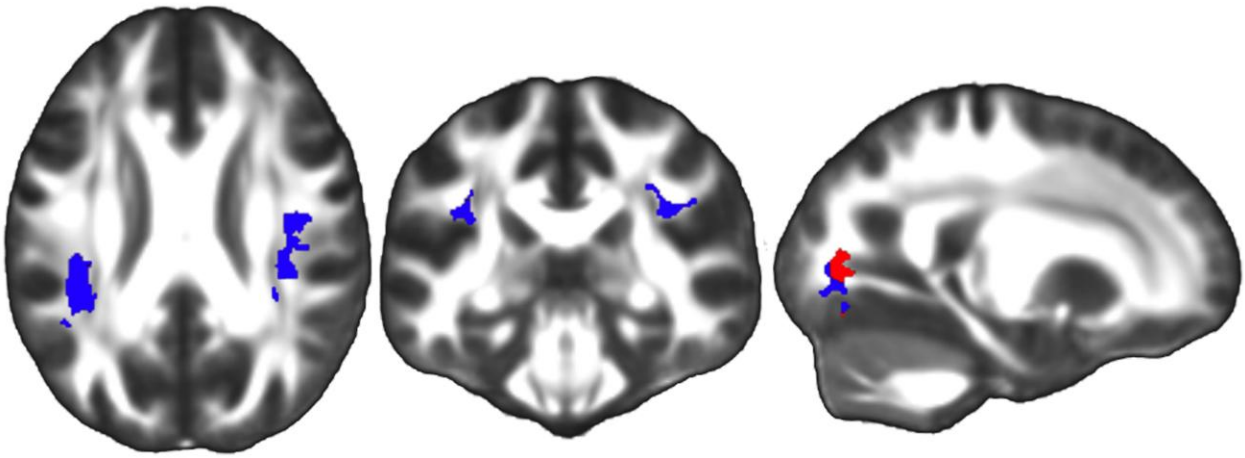


Figure 7: Linear regression analyses, in which neuropsychological test scores were the independent variables and FA maps were the dependent variables, demonstrated a significant correlation between performance on a finger tapping task and FA in the splenium of the corpus callosum among TBI patients at the second time point (red). The left side of the statistical map is patient left and right is patient right.

Figure 7

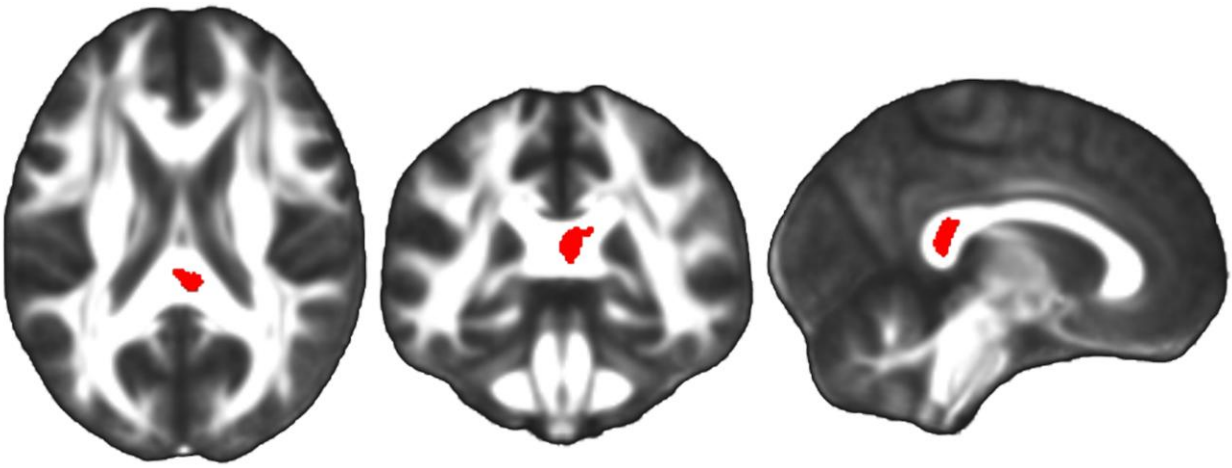


Figure 8: (a) Linear regression analyses demonstrated a significant positive correlation between TBI patients' performance on the Trails A visuomotor speed task and FA in bilateral regions of the sagittal stratum at the first time point (red), and in a unilateral region of the left sagittal stratum at the second time point (overlapping blue region). (b) A positive correlation was also observed between change in Trails A score between the first and third time points and change in sagittal stratum FA over the same four year study duration (red). The left side of the statistical map is patient left and right is patient right.

Figure 8

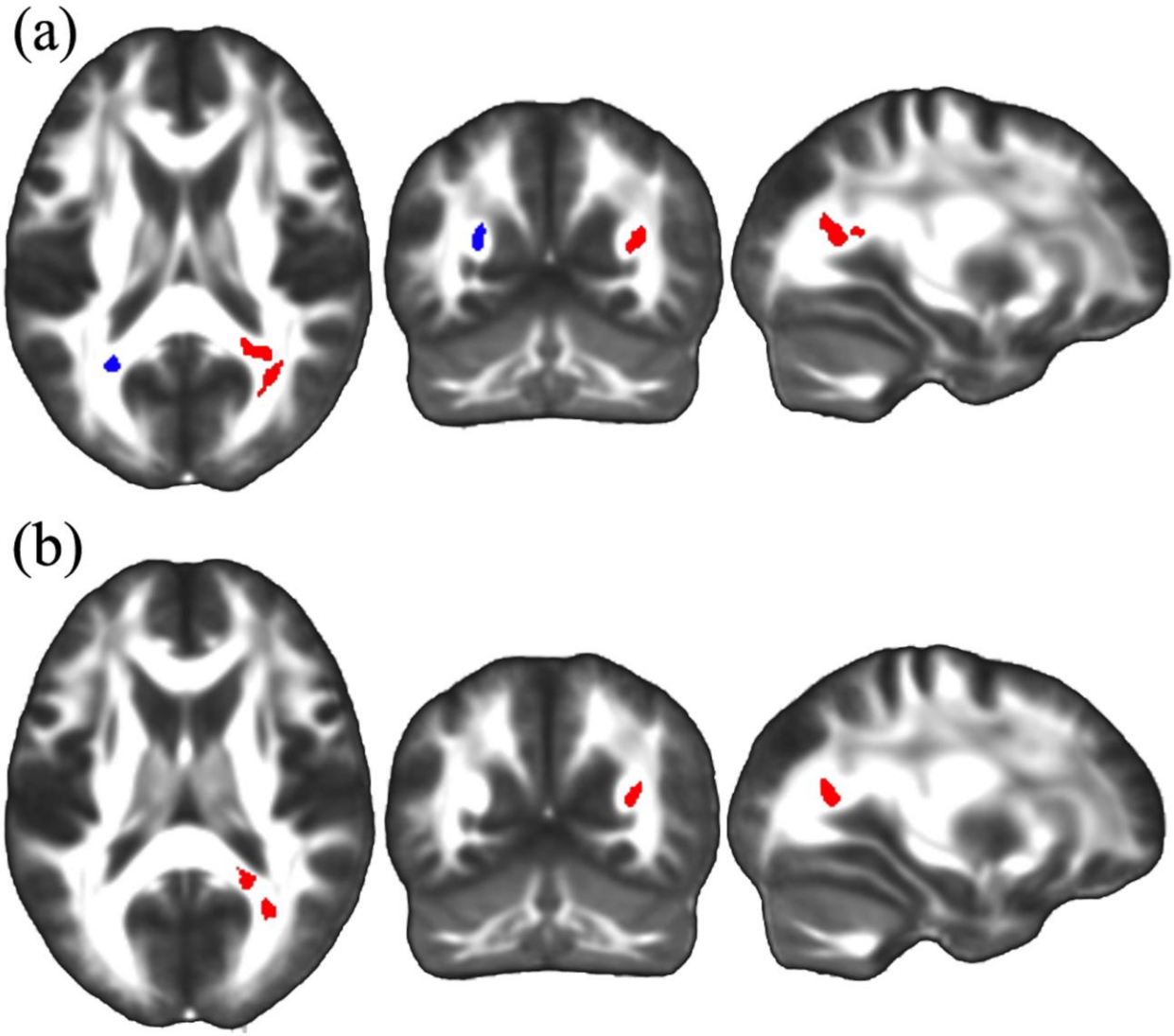
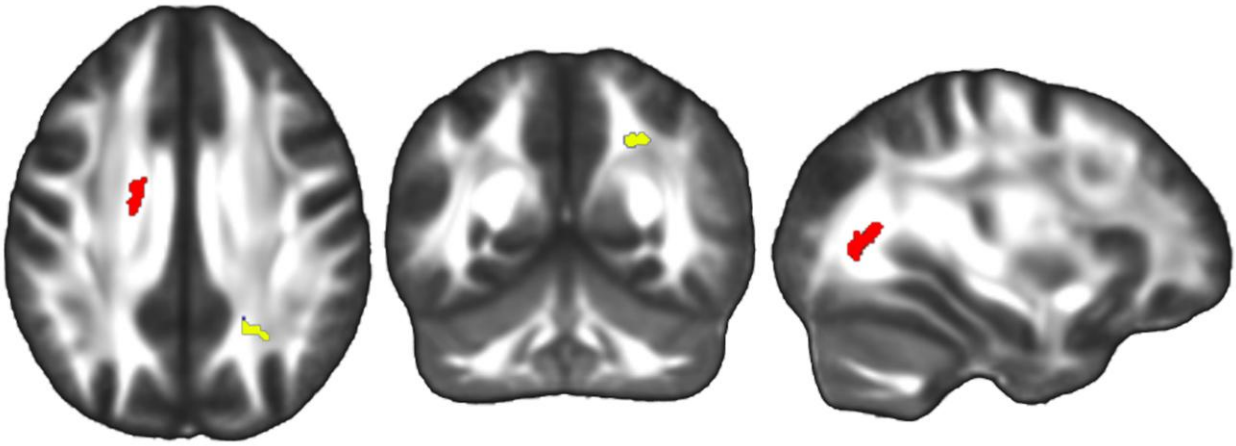


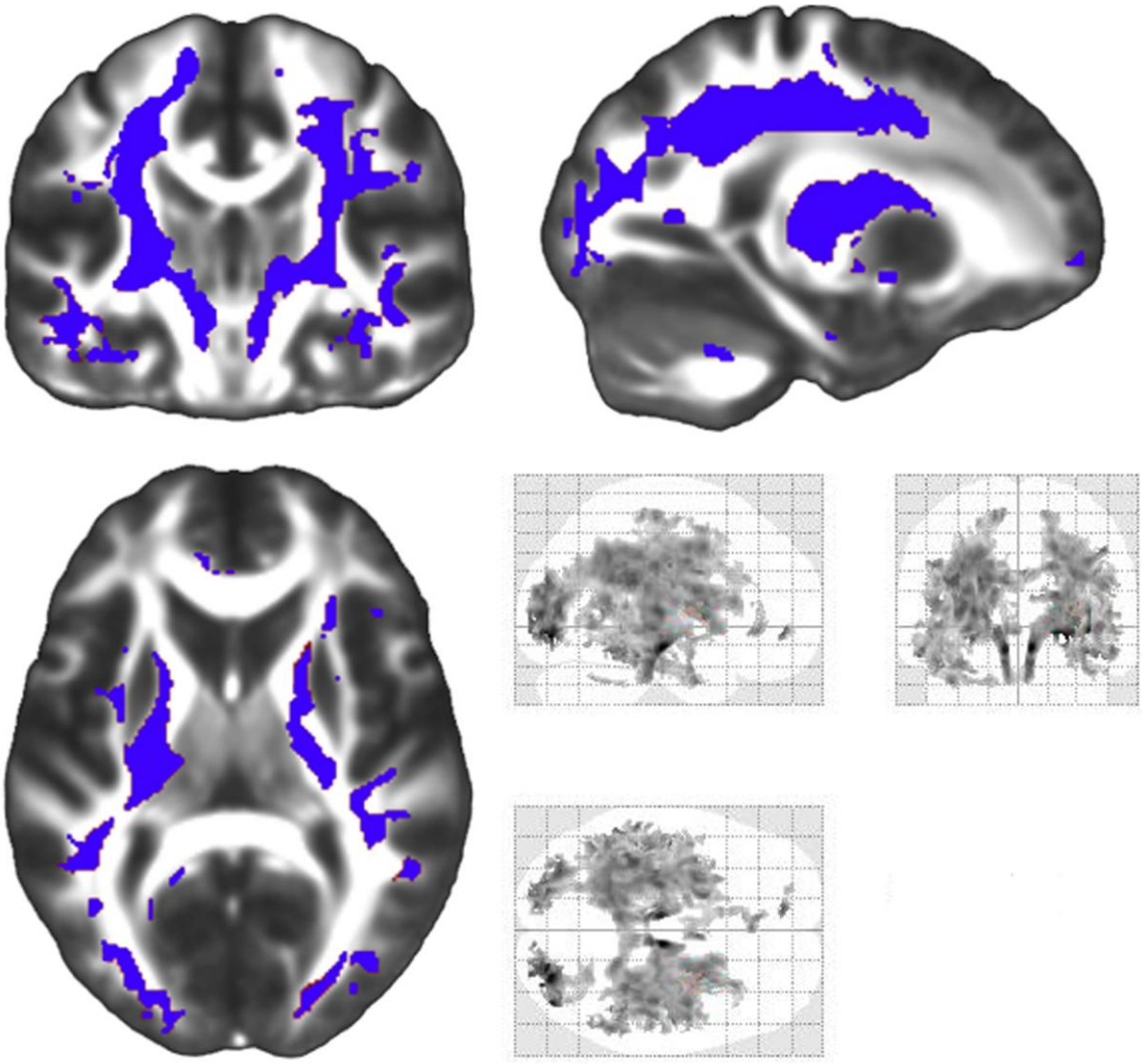
Figure 9: Linear regression analyses revealed a significant positive correlation between change in the cognitive component of the Trails B score between time one and time three and FA change in the left superior SLF and right optic radiation over the same four year study duration (red). Analyses of sub-intervals revealed there was also a significant positive correlation between change in the Trails B cognitive component between the first and second time points, and FA change in the right posterior SLF over the same one year first interval duration (yellow). The left side of the statistical map is patient left and right is patient right.

Figure 9



Supplementary Figure: A supplementary whole-brain analysis of radial diffusivity change among patients that was not restricted to regions demonstrating an FA effect was conducted. This analysis was carried out in the same radial diffusivity factorial model used in the restricted radial diffusivity analyses, and statistical thresholds used in FA analyses were employed. This analysis examined decreases in radial diffusivity during the four year study duration. Regions of decreased radial diffusivity included significant longitudinal decreases in radial diffusivity in superior and inferior longitudinal fasiculi, internal and external capsules, forceps major and minor and the descending corticospinal tract (blue). The tubular regions of change that extend through the descending corticospinal tract were not constrained on any side by the white matter mask employed in our analyses. The left side of the statistical map is patient left and right is patient right.

Supplementary Figure



Chapter 3:

Longitudinal volumetric changes following traumatic brain injury: A tensor based morphometry study

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Abstract

After traumatic injury the brain undergoes a prolonged period of degenerative change that is paradoxically accompanied by cognitive recovery. The spatiotemporal pattern of atrophy and the specific relationships of atrophy to cognitive changes are ill understood. The present study used tensor based morphometry and neuropsychological testing to examine brain volume loss in 17 TBI patients and 13 controls over a four year period. Patients were scanned at two months, one year and four years post-injury. High-dimensional warping procedures were used to create change maps of each subject's brain for each of the two intervals. TBI patients experienced volume loss in both cortical areas and white matter regions during the first interval. Furthermore, we observed continuing volume loss in extensive regions of white matter during the second interval. Neuropsychological correlations indicated that cognitive tasks were associated with subsequent volume loss in task-relevant regions. The extensive volume loss in brain white matter observed well beyond the first year post-injury suggests that the injured brain remains malleable for an extended period, and the neuropsychological relationships suggest that this volume loss may be associated with subtle cognitive improvements.

Introduction

For decades now it has been known that traumatic brain injury (TBI) is associated with initial gross decreases in brain volume due to contact and acceleration/deceleration, hemorrhage, and edema related injuries. Animal studies demonstrate that via a variety of mechanisms traumatic brain injury leads to both necrosis and apoptosis throughout the brain, which following removal of cellular debris by macrophages result in gross loss of brain tissue volume (Chen, Fong, Lee, & Chiu, 2011; Farkas & Povlishock, 2007; Kelley, Farkas, Lifshitz, & Povlishock, 2006; Kelley, Lifshitz, & Povlishock, 2007; Maxwell, Povlishock, & Graham, 1997; Povlishock, 1993; Povlishock & Christman, 1995; Takeuchi & Nawashiro, 2011). Results from animal studies have been convergent with findings in humans, both in studies using ex vivo immunohistochemical methods and in studies employing in vivo neuroimaging techniques (Bigler, Anderson, & Blatter, 2002; Bigler & Maxwell, 2011; Bombardier et al., 2010; Ding et al., 2008; Gale, Burr, Bigler, & Blatter, 1993; Gale, Johnson, Bigler, & Blatter, 1995; MacKenzie et al., 2002; Sidaros et al., 2009; Takeuchi & Nawashiro, 2011; Trivedi et al., 2007; Warner et al., 2010). Despite an extensive body of previous work indicating both initial volume loss and long-term cognitive change, there is a paucity of studies longitudinally examining brain change from the subacute to the chronic phase of recovery extending past one year post-injury. The present study investigates the specific pattern of regional volume loss beyond the first year post-injury and whether regional volumetric losses correlate with changes in cognitive and motor abilities.

Tensor Based Morphometry (TBM) has previously been shown to be sensitive to longitudinal volume changes induced by TBI (Kim et al., 2008; Sidaros et al., 2009). TBM derives a tensor map that reflects the amount of stretching or contraction needed to transform a

particular voxel in an initial brain image into the corresponding voxel in a later image of the same brain. The transformations, known as Jacobian determinants, are represented as a spatial map depicting where the brain has expanded or contracted over an interval (Leow et al., 2006). These Jacobian maps provide information concerning the progression of brain changes post-injury, and could be useful for patient monitoring and for assessing the effectiveness of drug or behavioral therapies.

The current study is an extension of prior work on this cohort of TBI patients. One previous report, Bendlin et al., (2008), included voxel-based morphometry (VBM) analyses of initial volumetric differences between TBI patients and controls as well volumetric changes during an interval spanning two months and one year post-injury. Results showed that patients exhibited both decreased initial volume and a greater magnitude of volume loss compared to controls in diffuse regions of gray and white matter over the interval. A related report on participants from this cohort, Trivedi et al. (2007), showed large-scale decline in total brain volume with concomitant improvements in cognition from baseline to one year follow-up. For the current study, we contacted the prior participants and invited them to take part in a third round of studies approximately four years post injury. We adopted the TBM methodology for this study in order to investigate the fine-grained longitudinal changes that occurred between three measurements at two months post injury, one year post injury and four years post injury. These detailed analyses provided novel longitudinal information beyond whole brain measurement, or VBM, reported in our prior papers and extend the follow up window from one year to four years.

The primary goal of the present study was to assess the nature and localization of volumetric contraction that occurs beyond the first year post-injury in TBI patients. The existing

study most similar to the present work, by Sidaros and colleagues (2009), used TBM to examine regional volume change during the first year post injury and demonstrated diffuse white matter contraction and clinical improvement during this one year interval. Based on this excellent work and other previous research indicating early volume loss in both diffuse regions of white matter and injury-specific regions of gray matter, (Levine et al., 2008; Siren et al., 2006), and subtle late phase (beyond the first year) cognitive recovery that is similar across patients with heterogenous injury localizations (V. Anderson, Godfrey, Rosenfeld, & Catroppa, 2011; Demir, Altinok, Aydin, & Koseoglu, 2006; Keren, Reznik, & Groswasser, 2001; Stern & Stern, 1985; Whitnall, McMillan, Murray, & Teasdale, 2006), we predicted that late phase volume loss would be similar across subjects and concentrated in brain white matter. Additionally, this study sought to investigate whether neuropsychological testing after injury could be used to predict spatial patterns of volume loss over time, and whether changes in regional brain volume correlated with changes in neuropsychological test performance. Specifically, we expected poorer performance on cognitive tasks to be associated with brain change in regions relevant to the tasks, and that impaired performance would predict volume loss as compromised task relevant regions progressively atrophied. This hypothesis was based on previous studies indicating that removal of damaged tissue and cellular debris is an important part of recovery from TBI (Clark et al., 2008; Johanson, Stopa, Baird, & Sharma, 2011; Liu, Chen, Dietrich, & Hu, 2008; Zhang et al., 2008).

Methods

TBI patients

Forty-six TBI patients participated in an initial MRI scan, thirty-six returned for a second visit. Twenty of these returned for an invited study extension and completed a third visit. Useable images were acquired in seventeen individuals at all three visits. Two subjects were excluded due to acquisition errors, and one more was excluded due to a second head injury sustained in a motor vehicle accident between visits two and three. The mean age of the final group was 34.5 ± 12.0 years at the beginning of the study; mean education was 12.9 ± 1.8 years. The final TBI group consisted of three women and fourteen men. The majority of the patients included in the study received acute treatment at the University of Wisconsin Hospital and Clinics level 1 trauma center and were referred from the departments of Neurosurgery, Trauma and/or Rehabilitation. The inclusion criteria for TBI consisted of involvement in a rapid impact injury to the brain (such as a motor vehicle accident or fall) causing a loss of consciousness, and subsequent admission for emergency medical attention. Patients sustaining penetrating or open head injuries were excluded from the study. Depressed skull fracture was not excluded (one patient). The average GCS score of patients at hospital admission was 7.2. For a detailed overview of clinical characteristics and injury descriptions, see Table 1. All TBI patients were less than 3 months post-injury at their first visit, and most were studied between 8 and 12 weeks post-injury, depending on their availability and other medical issues related to their injury. Exclusion criteria consisted of current major Axis I psychiatric disease or history of non-injury related major medical conditions (cancer, diabetes, or previously diagnosed neurological condition). All patients gave informed written consent under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board.

Healthy Controls

Thirty-six control participants were recruited from the community and from the University of Wisconsin campus via advertisement. Seventeen of these participants returned for two additional visits. Useable scans were acquired in thirteen participants at all visits (differences in acquisition necessitated the exclusion of four scans). The mean age of the final group was 26.8 ± 8.9 years at the beginning of the study; education was 15.2 ± 2.6 years; and there were five men and eight women. Exclusion criteria were identical to the TBI group, with the addition that head injury was also exclusionary for controls. MR scanning of control participants occurred on approximately the same schedule as that of TBI patients. All participants gave informed written consent under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board.

Procedures

Volunteers participated in three testing sessions, each consisting of neuropsychological testing and MR imaging. TBI patients were tested at three visits. Visit 1 was acquired approximately 2 months post injury (m= 75 days post injury, ranging from 25 days to 139 days). Visit 2 was acquired approximately one year post injury and slightly less than one year after Visit 1 (m= 395 days post-injury and 319 days after Visit 1, for an interval ranging from 226 days to 414 days). Visit 3 was acquired approximately four years post injury three years after Visit 2 (m= 1612 days post-injury and 1217 days after Visit 2, for an interval ranging from 956 days to 1651 days). Controls also participated in three visits with approximately one year (m= 249 days) between Visit 1 and Visit 2 (ranging from 74 days to 374 days) and three years (m= 1203 days) between Visit 2 and Visit 3 (ranging from 778 days to 2011 days). The difference in inter-scan duration was not significant between groups for either scan interval.

Neuropsychological Examination

On the day of each scan (at each of the three visits), a neuropsychological battery that included: COWAT (Controlled Oral Word Association Test), WRAT-III (Wide Range Achievement Test) Reading Subtest (an approximation of pre-injury intellectual attainment), Finger Tapping, WAIS-III Digit Span and Trail Making Test A and B was administered to each participant. These tests were selected based on previous research in our lab that suggested their probable relevance to TBI-induced behavioral changes. Analyses were conducted using raw scores and age, gender and education were included as covariates in the image analysis models. Participants were also administered several questionnaires, including a health history questionnaire, the BDI-II (Beck Depression Inventory), and the STAI (State-Trait Anxiety Inventory).

Magnetic resonance imaging

All participants underwent magnetic resonance imaging on a General Electric 3.0 T SIGNA (Waukesha, WI) MRI system with a quadrature birdcage head coil. Structural scans included an axial whole brain 3D T1-weighted inversion recovery-prepped spoiled gradient echo scan with the following parameters: inversion time = 600 ms, repetition time/echo time/flip angle = 9 ms/1.8 ms/20°; acquisition matrix = 256 × 256 × 124; field of view (FOV) = 240 mm; slice thickness = 1.2 mm (124 slices); receiver bandwidth = ± 16kHz; acquisition time = ~7.5 minutes. The reconstruction voxel size of 3D T1-weighted images was .94mm × .94mm × 1.2mm. A neuroradiologist (HR) reviewed all structural MR images to identify the location and extent of lesions associated with the TBI and to identify non-injury related brain abnormalities that might exclude subjects from the statistical analyses.

Image Preprocessing

To create the Jacobian maps used for TBM for each interval, the following steps were taken (Figure 1): (1) visit 1 T1-weighted (T1W) images and visit 3 T1W images were co-registered to visit 2 T1W images using Statistical Parametric Mapping (SPM8; Wellcome Department of Imaging Neuroscience, University College London, UK) linear rigid-body co-registration; (2) high-dimensional warping was used to produce warped images and Jacobian images of the deformations for each subject; (3) warped T1W images were averaged together; (4) the averaged T1W image was then normalized to the Montreal Neurological Institute (MNI) International Consortium for Brain Mapping (ICBM) template in SPM8; (5) the normalization parameters from this process were applied to the Jacobian images; (6) the Jacobians were smoothed with an 8-mm isotropic Gaussian (Figure 1). Visit 1 and visit 3 images were co-registered and warped to visit 2 images in order to minimize the effect of interval-related interpolation. The visit 3 to visit 2 Jacobian images were inverted such that change signified by these images was in the same direction as that signified in the visit 1 to visit 2 images (meaning both sets of Jacobians indicated atrophy). Smoothing was employed to facilitate inter-subject comparisons and enhance the signal to noise ratio.

Statistical Analyses

Statistics assessing demographic differences and differences in neuropsychological task performance between groups and within groups over time were carried out in SPSS19. Differences in mean age and education were assessed using independent-samples two-tailed t-tests, differences in group gender proportions were assessed using a chi-squares test, between-groups differences in neuropsychological task performance were calculated using independent-

samples two-tailed t-tests, and within-groups differences in neuropsychological task performance over time were calculated using paired-samples two-tailed t-tests.

TBM statistical tests were performed on Jacobian maps using Factorial ANOVA models in SPM8. We hypothesized that there would be group differences in brain volume loss during both the first and second interval, and that the specific pattern of volume loss within the TBI group would be different during the first interval compared to the second. This was tested using a factorial design with interval (Interval 1 Jacobian maps; Interval 2 Jacobian maps) as the first factor and group (TBI, Control) as the second factor. We hypothesized that there would be overall group differences in patterns of volume loss and that these differences would vary between the two intervals, manifesting as a significant interaction between group and interval. We also hypothesized that the TBI group would demonstrate significant regional volume contraction compared to the control group during both the first and second interval, and that the pattern of this volume change within the TBI group would be different in the first compared to the second interval. Simple effects analyses within the Factorial ANOVA model were used to investigate between-groups differences in patterns of volume loss during each interval. Paired samples t-tests were used to investigate within-groups differences in patterns of volume loss between the two intervals. Percentage volume loss in key regions (as shown in Figure 4) was calculated by extracting the values of 3mm spherical ROIs at peak points within key regions at the same coordinates for each subject and subsequently averaging values across groups and converting them into percentage change.

Total brain volume was calculated by using SPM to segment individual T1W images into gray matter, white matter and CSF, and then adding the gray and white matter measurements. Percentage change in brain volume between time points was calculated by subtracting earlier

volumes from later volumes, and then dividing the result by the initial volume. Between groups differences in percentage of brain volume loss and annual rates of brain volume loss were calculated using independent samples two-tailed t-tests in SPSS 19. Within-groups differences in percentage brain volume loss and annual rates of brain volume loss were calculated using paired-samples two-tailed t-tests in SPSS 19.

Our hypotheses regarding correlations with neuropsychological test scores were investigated using linear regression implemented in SPM8, where test scores were independent variables and Jacobian maps were dependent variables. Age, gender, education (in years) and interval length (in days) were used as covariates in all models. A False Discovery Rate (FDR) of $p<.05$ was used in all statistical maps, and the cluster threshold was set at 500 contiguous voxels. This approach has been used in multiple previous studies (Hua et al., 2008; Leow et al., 2009; Tao et al., 2009). For descriptive purposes, numerical correlations between structural change and neuropsychological task performance were calculated in regions shown to be significant in our SPM analyses. Correlational values were calculated by extracting the values of 3mm spherical ROIs at peak points within regions at the same coordinates for each subject and subsequently averaging values across groups and converting them into percentage change. The percentage change values were then correlated with task scores or changes in task scores. We also sought to investigate whether correlations existed between total brain volume change and neuropsychological task performance. We investigated whether neuropsychological task performance was predictive of subsequent rates of total brain atrophy. Correlations between neuropsychological measures and whole brain volume changes were carried out in SPSS 19.

Results

Demographic and behavioral results

Group demographics are shown in Table 1. There was no significant age difference between TBI patients and controls. Controls had more education than TBI patients (t=2.76; df=20.49; two-tailed p=.012). In addition, there was a significantly lower proportion of females in the TBI group compared to the control group ($\chi 2(1,30)=6.111$, p=.0134). Patient injury characteristics are also shown in Table 1. Neuropsychological test performance summaries are shown in Table 2. TBI patients performed significantly worse than controls on a number of tests at Visit 1 (DSPAN, Trails A, Trails B, COWAT) and one test at Visit 3 (Trails A). There were no significant between groups differences at Visit 2. TBI patients did not differ significantly from controls on measures of pre-morbid intelligence (WRAT-III Reading Subtest) or emotional functioning (BDI-II, STAI-Trait and STAI-State).

TBM results- Interaction

All imaging reports are bilateral unless otherwise noted. The results of the group by interval interaction produced significant clusters in large surface regions of all four major cortices (frontal, temporal, occipital and posterior/inferior parietal) as well as a medial inter-hemispheric cortical region, in medial and right lateral cerebellum, and in white matter regions including anterior and superior corona radiata, superior longitudinal fasciculus, posterior inferior longitudinal fasciculus, genu and splenium of the corpus callosum, forceps minor, right internal capsule, superior external capsule and sagittal stratum (Figure 2).

TBM results- Simple Effects

TBI patients demonstrated greater volume loss than control subjects in the following regions during the first interval: left frontal, temporal, posterior parietal, occipital and inter-hemispheric cortices, parahippocampal gyrus, lateral cerebellum, brainstem, thalamus, external capsule and splenium of corpus callosum (Figure 3, Figure 4). TBI patients also demonstrated greater volume loss than control subjects in the following regions during the second interval: left parahippocampal gyrus, brainstem, thalamus, inferior longitudinal fasciculus, superior longitudinal fasciculus, external capsule, forceps minor, superior and anterior corona radiata and splenium, body and genu of the corpus callosum (Figure 3, Figure 4). There were no brain regions where the control group showed significantly greater volume loss than the TBI group during either interval. Paired samples t-tests revealed that there were no significant differences between the pattern of volume loss observed in the first interval compared to the second interval within the TBI group. Analogous paired-samples t-tests in the control group also failed to yield significant clusters.

Total Brain Volume Loss

Independent-samples two-tailed demonstrated that there were no significant differences in the total amount of brain volume loss nor in the annual rate of whole brain volume loss between the two groups during either interval (Interval 1 Total: TBI patients- 2.1% ± 4.6%; Controls- 0.2% ± 6.9%; Interval 1 Annual: TBI patients- 2.8% ± 5.4%; Controls- 0.2% ± 2.5%; Interval 2 Total: TBI patients- 3.3% ± 3.6%; Controls- 1.3% ± 2.5%; Interval 2 Annual: TBI patients- 1.1% ± 1.1%; Controls 0.4% ± 0.8%). During the entire four year study period, however, TBI patients demonstrated significantly greater total volume loss than controls. (TBI: 5.4% ± 3.3%, Control: 1.2% ± 2.5 %, p= .0006). The average annual volume loss for TBI

patients during the four year study period was also significantly greater than that of controls (TBI: 1.3% ± 0.7%, Control: 0.3% ± 0.7%, p= .0007). Paired-samples t-tests revealed that there were no significant within-groups differences in total or annual whole brain volume loss between the two intervals studied. All whole brain volume results are reported in Table 3.

Neuropsychological correlations

Of the six neuropsychological measures included in the study, two held some predictive value for volume loss among TBI subjects. Slower completion speed on the Trails A visuomotor speed task at Visit 1 predicted greater volume loss in brainstem and cerebellum, both regions relevant to the task, during both the first interval and the second interval (Brainstem 1: r=.762; p=.00037; Cerebellum 1: r=.792, p=.00015; Brainstem 2: r=.880, p=.000003; Cerebellum 2: r=.755; p=.000001) (Figure 5). The second task that demonstrated predictive value for volume loss in TBI patients was Trails B. This tasks requires subjects to trace a path connecting alternating letters and numbers as quickly as possible (i.e. 1, A, 2, B, 3, C, etc.). Although there was no correlation between volume loss and Trails B performance during the first interval, slower completion speed of Trails B at Visit 2 (one year post injury) predicted subsequent volume loss in the anterior cingulate cortex during the second interval (r=.856; p=.00001) (Figure 6).

Correlations with whole brain volume loss demonstrated that among TBI patients, poorer scores on DSPAN, Trails A and Trails B at the first time point were associated with greater subsequent whole brain volume loss during the first interval, Trails B and COWAT scores from the second time point were associated with greater subsequent whole brain volume loss during the second interval, and none of the first time point cognitive task scores were associated with greater whole brain volume loss during the four year study duration. There were no significant

correlations between neuropsychological test performance and subsequent whole brain volume loss among controls. Additionally, improvement in Trails A and Trails B scores during the first interval correlated with greater concurrent whole brain volume loss during the same interval among TBI patients. There were no significant correlations between change in neuropsychological task performance during the second interval and total brain volume loss during the same period, nor were there significant correlations between change in neuropsychological task performance and total brain volume loss during the entire study duration. For a complete review of whole brain volume correlations see Table 4.

Discussion

We studied longitudinal brain atrophy occurring from two months to one year and one year to four years post-injury in TBI patients, using TBM to evaluate the presence of regional contraction. The results of the group by interval interaction indicate that there was inter-interval dissimilarity in group-wise differences in volumetric contraction in large regions of all four major lobes (frontal, temporal, occipital and posterior parietal) as well as a medial inter-hemispheric cortical region, in medial cerebellum, and in white matter regions including anterior and superior corona radiata, superior longitudinal fasciculus, posterior inferior longitudinal fasciculus, corpus callosum, forceps minor, left internal capsule, external capsule and sagittal stratum. Combining this result with our simple effects results (which demonstrate that different sets of regions exhibited greater contraction among TBI patients compared to controls in the first versus the second interval) indicates that while some regions exhibited contraction that was greatest during the first interval (such as the parietal and occipital cortices); other regions demonstrated contraction that was greatest during the second interval (such as white matter of the superior and inferior longitudinal fasciculi).

Whole brain volumetric analyses indicated that while TBI patients did not exhibit greater volume loss than controls during either interval examined independently, patients did exhibit both greater total volume loss and a higher annual rate of volume loss during the four year study duration. These findings, combined with the factorial models, support our hypothesis that volumetric contraction continues to occur in TBI patients at a level in excess of that observed in controls beyond the first year post-injury, and that late chronic phase atrophy is localized differently than volume loss observed during the first year.

We found that TBI patients experienced a greater rate of atrophy than controls in frontal, temporal, posterior parietal, occipital and inter-hemispheric cortices, lateral cerebellum, brainstem, thalamus, parahippocampal gyrus, external capsule and splenium of corpus callosum during the first year post-injury. This pattern of volumetric contraction that encompassed both gray and white matter is consistent with previous work indicating that TBI patients as a whole experience a common pattern of atrophy as well as multi-focal cortical changes, (Bendlin et al., 2008; Ding et al., 2008; Kiraly & Kiraly, 2007; Sidaros et al., 2009; Trivedi et al., 2007).

Animal studies and ex vivo immunohistochemical studies in humans show that a number of necrotic and apoptotic processes (calcium influx caused by initial insult and secondary edema, edema-induced cytoskeletal disassembly, early and late axonal disconnection) as well as extensive Wallerian degeneration occur during the sub acute period and first year following TBI (Engel et al., 2000; Farkas & Povlishock, 2007; Fox & Faden, 1998; Gale et al., 1995; Kelley et al., 2006; Kelley et al., 2007; Lifshitz, Kelley, & Povlishock, 2007; Singleton, Zhu, Stone, & Povlishock, 2002). These processes represent potential mechanisms for contraction observed in our study.

The second interval (between one and four years post-injury) was characterized by diffuse and extensive white matter atrophy in TBI patients and a relative lack of cortical contraction. Specifically, we observed significantly greater contraction in TBI patients compared to controls in brainstem, parahippocampal gyrus, thalamus, inferior longitudinal fasciculus, superior longitudinal fasciculus, internal capsule, external capsule, forceps minor, superior and anterior corona radiata and splenium, body and genu of the corpus callosum. Based on previous work indicating that the TBI patients continue to exhibit subtle cognitive improvements for many years after injury and other previous work demonstrating that regional white matter

microstructural improvements in TBI are co-occurring with volume loss, it is conceivable that the volumetric contraction in white matter may be at least partially reorganizational or adaptive (Cernich, Kurtz, Mordecai, & Ryan, 2010; Dikmen et al., 2009). This is a hypothesis for future work. Alternatively, there is a recent hypothesis that TBI is not merely an event but rather the initiation of a disease state with lifelong systemic impacts (Masel & DeWitt, 2010). Continuing white matter atrophy may confer risk for post-trauma psychiatric diseases, metabolic disorders or neuroendocrine dysregulation, all of which are commonly associated with TBI (C. V. Anderson, Bigler, & Blatter, 1995; Bombardier et al., 2010; Dikmen et al., 2009; Kiraly & Kiraly, 2007; Whitnall et al., 2006). Regardless of the exact causes of atrophic changes, these novel data support an emerging picture of continuing long-term change that may inform treatment and rehabilitation in TBI.

Our hypothesis that impaired performance on neuropsychological measures (presumably caused by damage to brain regions necessary to complete the tasks) would predict volume loss in task relevant regions among TBI patients was partially supported by results of the Trails A and Trails B correlations. We theorize that other tasks tested for predictive value failed to produce significant correlational results because the other tasks tested are not as sensitive to TBI-induced neurological and cognitive changes as the Trails tasks, and therefore were not associated with specific undetectable initial damage (Frencham, Fox, & Maybery, 2005). Slower completion speed on Trails A at the initial visit predicted atrophy in the white matter of the brainstem and cerebellum in both the first and second intervals (Figure 5), likely reflecting common downstream effects secondary to diffuse and variable injury in the cerebrum and cerebellum that coalesce in these dense white matter areas (middle cerebellar peduncle and cortical spinal tract). At the second visit, slower completion speed on the Trails B task, which requires subjects to

engage in set shifting, was associated with volume loss in the anterior cingulate (a structure known to be required in set shifting and mental conflict resolution) during the subsequent interval (Figure 6). Whether this is related to gradual volume loss as cellular debris was slowly cleared away, or a plasticity-related process cannot be determined by these data, and would be an interesting topic for further research.

In addition to region specific correlations between regional volume loss and the Trails A and Trails B tasks, poorer performance on each of these tasks at visit 1 was associated with greater whole brain volume loss during the subsequent year. While it is difficult to interpret the precise meaning of these findings from imaging analyses alone, these results do underscore the sensitivity of the Trails A and B tasks to TBI induced damage. Other correlations between neuropsychological task performance and subsequent whole brain volume loss included visit 1 DSPAN performance and visit 2 Trails B and COWAT performance. No such correlations were observed among controls, indicating that the association between poorer task performance and subsequent whole brain volume loss is specific to TBI patients, and that these tasks may be sensitive to damage not easily observed on initial MRI.

Limitations

Some limitations of this study deserve mention. One limitation is that gender composition and education level differed between the two groups, possibly affecting results. Ideally, patients should be compared to matched controls; however, the TBI population is generally more heavily male and less educated than the general population, and previous studies of volumetric change in TBI patients that use gender and/or education matched controls (Kim et al., 2008; Sidaros et al., 2009) and those that use controls that are not thusly matched (Bendlin et al., 2008; Trivedi et al., 2007) return similar results. Furthermore, we included gender and education as covariates in our

statistical models to decrease the effect of these differences. While there was no significant difference in the age distributions of the two groups, there is a trend toward a group difference. Additionally, the results of this study may be limited by the small size of the final groups. The TBI population and college aged controls are both itinerant populations, and therefore difficult to track for long periods of time. While statistical analyses indicated there were not significant differences in age, education or injury severity within either group between those who dropped out of the study and those who completed all three visits, the high attrition in both groups could still have biased our results. Finally, while we employed a voxel-wise method for correction for multiple comparisons, this was done within contrasts only and not across the multiple contrasts performed.

Conclusion

This study demonstrates that TBI patients experience diffuse atrophy that is far in excess of age-matched controls for at least four years after injury. Several studies have shown that volume loss occurs in the first year following TBI, however this study is among the first to show that contraction occurs well beyond the first year post-injury. Our results show that, as opposed to widespread volume loss observed in the first year post-injury, late-phase volume loss is centered almost exclusively in brain white matter. This late-phase white matter volume loss is pervasive, as most large white matter tracts in the brain are affected. Furthermore, our neuropsychological results indicate that volume loss following TBI should be considered in a broader context of ongoing neurological and functional change. Future work combining neuropsychological testing, morphometry, and potentially fMRI, as well as work in animal models, over an extended follow-up period will yield greater insight into the mechanisms underlying cognitive changes following TBI.

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Table 1: *Individual level subject demographics for patients and controls and select patient injury characteristics*

Patients

Subject	Age	Education	Sex	GCS0	GCS24	Hrs15	Injury Notes
1	19	12	M	3T	7	334	DAI
2	30	12	M	3	7	217	Contusions, epidural hematoma, shearing of splenium
3	37	16	M	14	15	1	DAI , subarachnoid hemorrhage
4	18	11	M	11	11	76	Contusions, subarachnoid hemorrhage
5	23	12	M	15	7	541	Contusions, skull fracture, subdural hematomas
6	41	16	F	3T	10	444	Contusions, subarachnoid bleeding, subdural hematoma
7	19	14	F	3T	11	91	Subarachnoid hemorrhage, subdural hematoma
8	24	14	M	3	7	662	DAI, contusions, epidural hematoma, subarachnoid hemorrhage
9	48	12	M	14	15	4	Subdural hematoma
10	39	10	M	3T	6	835	Extensive contusions, subdural hematoma
11	25	12	M	15	15	0	Multiple skull fractures, subdural hematoma, contusions
12	49	12	M	3T	8	110	Epidural hematoma, subarachnoid hemorrhage
13	45	12	M	3T	14	270	Skull fracture, frontal contusion
14	29	13	M	3	14	179	DAI, contusions, skull fracture
15	52	16	F	11	13	97	Skull fracture, subarachnoid hemorrhage, subdural hematoma
16	51	12	M	7	7	726	Depressed skull fracture, subdural and epidural hematomas
17	37	14	M	9	7	116	DAI, subarachnoid hemorrhage, subdural hematoma, shearing
Mean (SD)	35.4 (12.0)	12.9 (1.8)	82% m	7.2 (5.0)	10.2 (3.5)	276.7 (269.7)	

Controls

Subject	Age	Education	Sex
18	20	17	M
19	22	19	F
20	22	15	M
21	18	13	F
22	21	16	F
23	49	12	F
24	29	13	F
25	27	16	F
26	25	12	F
27	19	16	F
28	36	20	M
29	36	16	M
30	24	13	M
Mean (SD)	26.8 (8.9)	15.2 (2.6)	38% m
p	0.062	.012*	.013*

Note: Age of participants indicates the mean age of participants at the start of the study. P-values are based on the results of two-tailed independent-samples t-tests for age and education and a chi-squares test for gender proportions. Abbreviations are as follows: GCS0= Glasgow Coma Scale score at hospital admission, GCS24= Glasgow Coma Scale score 24 hours post-injury, Hrs15= Number of hours before patient reached a GCS score of 15, Scan1= Number of days between injury and initial scanning, DAI= Diffuse axonal injury, T= Patient was intubated at the time of GCS assessment.

Table 2: *Neuropsychological test performance*			
	Time 1 n, **Mean** (SD)	**Time 2** n, **Mean** (SD)	**Time 3** n, **Mean** (SD)
TBI Patients			
WRAT-III (Reading)	15, **47.4** (5.9)	17, **46.5** (5.3)	11, **47.3** (6.1)
DSPAN	16, **15.4****† (4.2)	17, **16.9** (4.1)	11, **16.6** (4.7)
TRAILS A (seconds)	17, **40.2***† (21.3)	17, **29.48**‡ (8.1)	12, **26.8**^{*o} (5.3)
TRAILS B (seconds)	16, **86.6***† (42.3)	17, **70.9**‡ (26.5)	12, **63.9**^{o} (37.6)
COWAT	17, **28.7*****† (11.1)	17, **36.2** (11.7)	12, **36.2**^{o} (12.5)
FT DOM	15, **46.2** (14.0)	17, **46.4** (6.9)	12, **45.6** (6.8)
FT NON	14, **42.1** (11.8)	16, **43.5** (8.8)	11, **42.5** (7.4)
BDI-II	17, **9.6**† (6.2)	17, **6.6** (5.9)	17, **7.8** (6.6)
STAI-T	16, **29.8**† (7.7)	15, **26.1** (7.5)	14, **27.2** (5.9)
STAI-S	16, **32.9**† (7.1)	16, **30.2** (7.3)	16, **30.7** (7.2)
Controls			
WRAT-III (Reading)	12, **50.5** (4.4)	8, **50.1** (5.7)	9, **51.7** (5.1)
DSPAN	12, **19.8** (3.5)	8, **20.3** (4.1)	9, **20.6** (5.2)
TRAILS A (seconds)	12, **26.3** (5.7)	8, **25.1** (5.5)	9, **21.1** (5.2)
TRAILS B (seconds)	12, **56.6** (27.8)	8, **54.1**‡ (12.6)	9, **41.2** (10.5)
COWAT	12, **54.0**† (6.4)	8, **45.2** (8.4)	9, **43.8**^{o} (12.5)
FT DOM	9, **44.6** (8.9)	8, **49.0** (6.6)	9, **48.5** (6.8)
FT NON	9, **41.8** (6.4)	8, **46.3** (6.7)	9, **47.3** (5.7)
BDI-II	12, **7.9** (10.1)	9, **7.1** (7.2)	13, **6.2** (6.0)
STAI-T	12, **30.2** (7.2)	8, **30.8** (7.1)	13, **32.1** (8.4)
STAI-S	12, **37.9** (13.9)	8, **38.8** (14.8)	12, **34.5** (12.1)

Note: Means are of raw scores. Between-groups differences were calculated using independent samples one-tailed t-tests, and significance levels are *p<.05, **p<.01, ***p<.001. Between groups differences are noted by the patient average scores only. Within-groups differences were calculated using paired samples two-tailed t-tests and a significance level of p<.05 was used. Differences between time 1 and time 2 are denoted by †, differences between time 2 and time 3 are denoted by ‡, and differences between time 1 and time 3 are denoted by ^{o}. Because there were missing values the sample sizes in the paired tests varied. Test abbreviations are as follows: WRAT-III (Reading)= Wide Range Achievement Test III Reading Subtest-raw score, DSPAN= Digit Span Test from the Weschler Adult Intelligence Scale, Third Edition, Trails A= Trail Making Test A, Trails B= Trail Making Test B,, COWAT= Cognitive Oral Word Association Test, FT DOM= Dominant Hand Finger Tapping Test, FT NON= Non-Dominant Hand Finger Tapping Test, BDI-II= Beck Depression Inventory, STAI-S= State-Trait Anxiety Test state score, STAI-T= State-Trait Anxiety Test trait score.

Table 3: *Annual and total percentage brain volume loss*						
	Time 1 to 2		Time 2 to 3		Time 1 to 3	
	Annual	Total	Annual	Total	Annual	Total
TBI	**2.8** (5.4)	**2.1** (4.6)	**1.1** (1.1)	**3.3** (3.6)	**1.3** (0.7)*	**5.4** (3.3)*
Control	**0.2** (6.9)	**0.2** (2.5)	**0.4** (0.8)	**1.3** (2.5)	**0.3** (0.7)	**1.2** (2.5)

Note: Bold numbers indicate mean volume loss and numbers in parentheses indicate the standard deviation of the mean. "Total" indicates total volume loss during the specified period and "Annual" indicates annual volume loss during the specified period. Independent samples two-tailed t-tests were used to determine between-groups differences on all measures included in the table, and significance levels are denoted *$p<.05$.

Table 4: *Neuropsychological test performance and subsequent whole brain volume loss*					
	WRAT-III (Reading) n, r	DSPAN n, r	TrailsA n, r	TrailsB n, r	COWAT n, r
TBI					
Interval 1	15, -.284	16, -.636**	17, .629**	17, .527*	17, -.199
Interval 2	17, -.134	17, -.178	17, -.523	16, .616**	17, -.647**
Total Study	17, -.495	16, -.451	17, .394	16, .166	17, -.152
Control					
Interval 1	12, -.225	12, .157	12, -.410	12, .268	12, .055
Interval 2	8, -.130	8, -.487	8, -.307	8, .105	8, -.190
Total Study	12, -.430	12, -.433	12, -.346	12, .318	12, -.052

Note: Interval 1 whole brain volume loss was tested for correlation with neuropsychological task performance at the first time point, Interval 2 whole brain volume loss was tested for correlation with neuropsychological task performance at the second time point, and Total Study (Interval 1 and Interval 2 combined) whole brain volume loss was tested for correlation with neuropsychological task performance at the first time point. Significance levels are denoted as follows: are *p<.05, **p<.01, ***p<.001. Test abbreviations are as follows: WRAT-III (Reading)= Wide Range Achievement Test III Reading Section, DSPAN= Digit Span Test from the Weschler Adult Intelligence Scale, Third Edition, Trails A= Trail Making Test A (motor), Trails B= Trail Making Test B (motor and cognitive), COWAT= Cognitive Oral Word Association Test.

Figures

Figure 1: Shows a schematic of image processing steps used to create Jacobian maps. Boxes containing processing steps are numbered and in white text, while boxes containing sets of images are in black text.

Figure 1

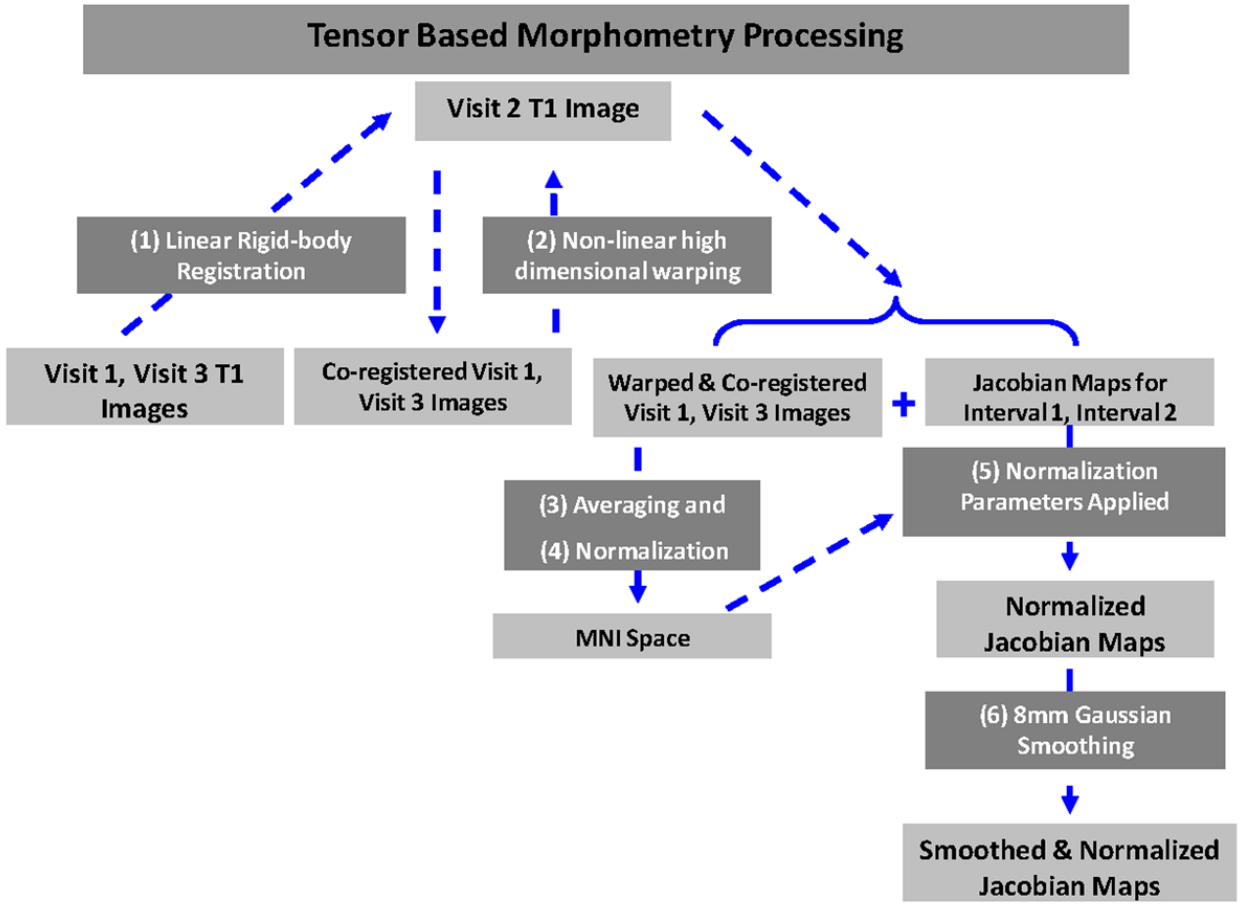

Tensor Based Morphometry Processing
Visit 2 T1 Image
(1) Linear Rigid-body Registration
(2) Non-linear high dimensional warping
Visit 1, Visit 3 T1 Images
Co-registered Visit 1, Visit 3 Images
Warped & Co-registered Visit 1, Visit 3 Images
Jacobian Maps for Interval 1, Interval 2
(5) Normalization Parameters Applied
(3) Averaging and
(4) Normalization
MNI Space
Normalized Jacobian Maps
(6) 8mm Gaussian Smoothing
Smoothed & Normalized Jacobian Maps

Figure 2: A significant interaction between Group and Interval was observed within the Factorial ANOVA model. There were inter-interval dissimilarities in group-wise differences in volumetric contraction in all regions shown in red. A False Discovery Rate (FDR) of $p<.05$ was utilized (corresponding to an F statistic of greater than 8.086), and a cluster threshold was set at 500 contiguous voxels.

Figure 2

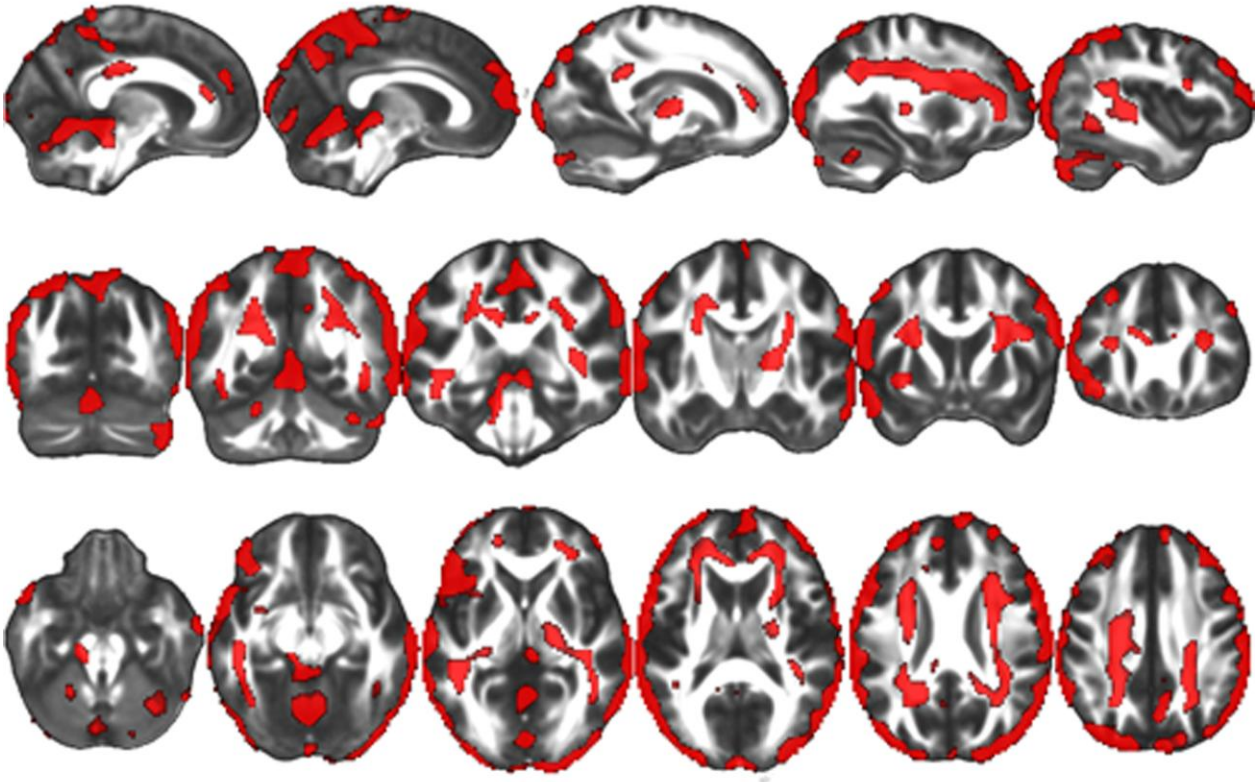


Figure 3: The TBI group demonstrated significantly more volume loss than the control group during both the first interval (shown in red) and the second interval (shown in blue). Yellow signifies regions where TBI subjects demonstrated significantly more contraction during both intervals. A False Discovery Rate (FDR) of $p<.05$ was utilized (corresponding to a t statistic of greater than 2.659 in the first interval contrast and 2.624 in the second interval contrast), and a cluster threshold was set at 500 contiguous voxels.

Figure 3

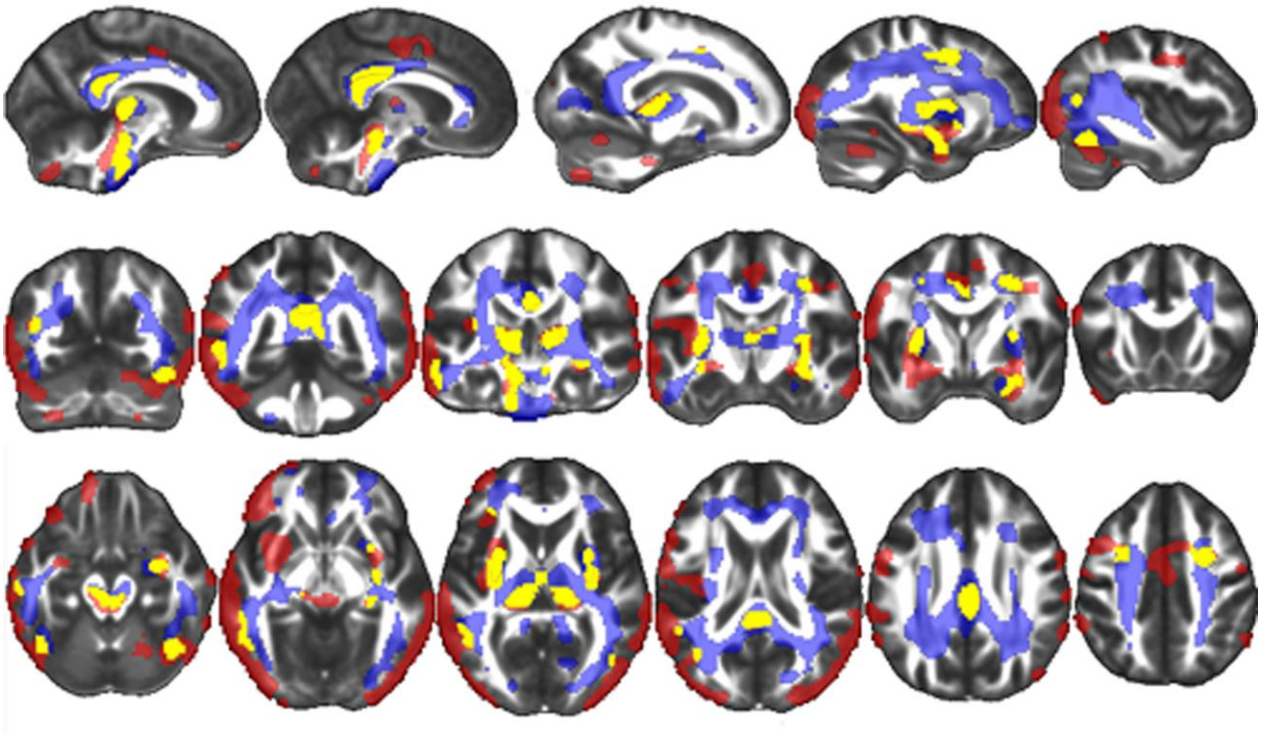


Figure 4: To demonstrate the degree of volume loss exhibited by TBI patients in different structures, values were extracted from 3mm spherical ROIs in several key regions at the same coordinates in each subjects' Jacobian maps. These values were converted to percentages and averaged. Second interval volume loss is shown as a percentage change from total volume at visit 2, and therefore total volume loss in a structure within a group for the study duration is equal to the sum of volume loss in the structure during each of the two intervals. ILF= Inferior longitudinal fasiculus, SLF= superior longitudinal fasiculus. Control 1= average percentage regional volume change among control participants during interval 1, Control 2= average percentage regional volume change among control participants during interval 2, TBI 1= average percentage regional volume change among TBI patients during interval 1, TBI 2= average percentage regional volume change among TBI patients during interval 2.

Figure 4

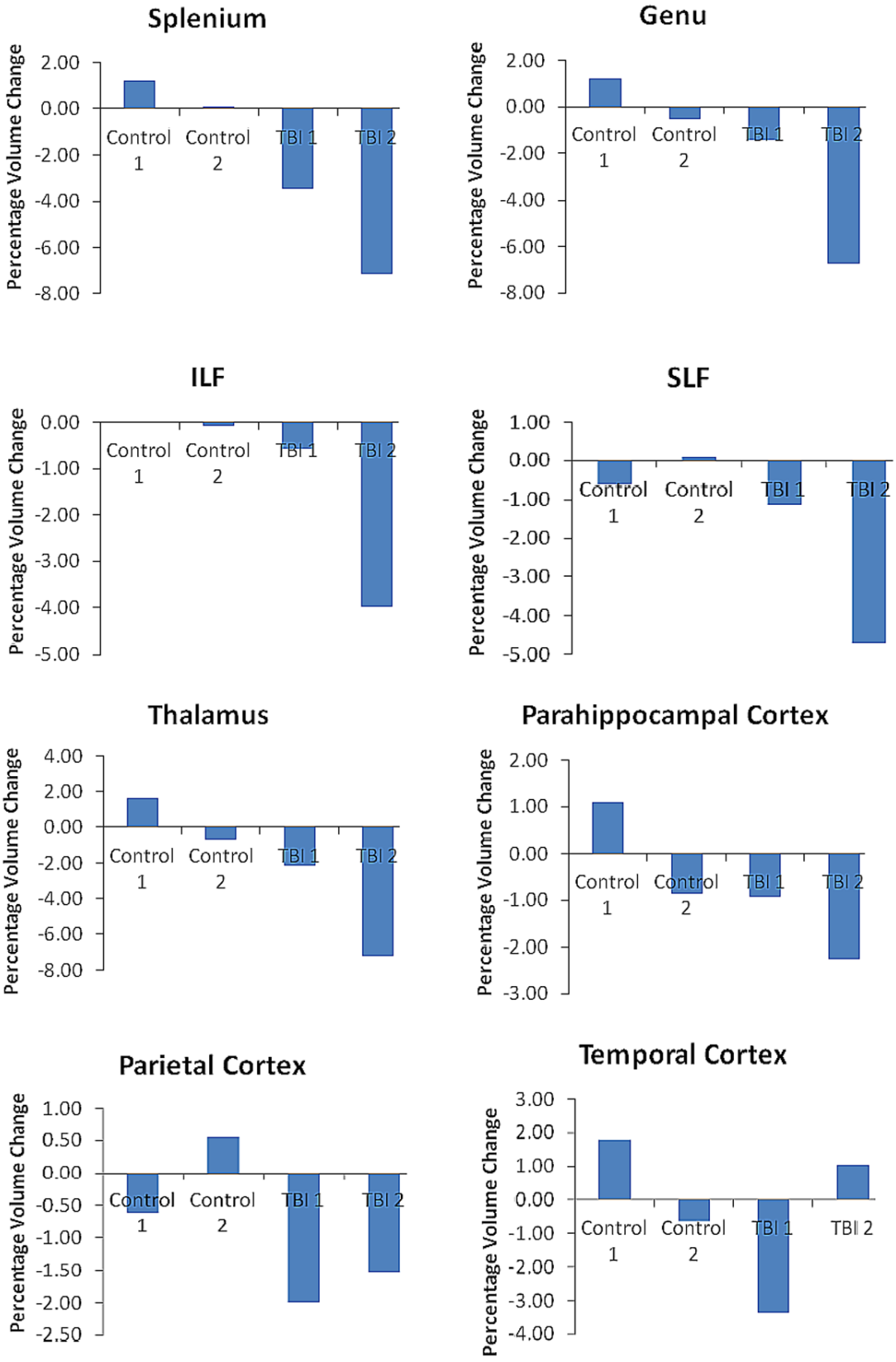

Splenium
Percentage Volume Change
2.00
0.00
-2.00
-4.00
-6.00
-8.00
Control 1
Control 2
TBI 1
TBI 2
Genu
Percentage Volume Change
2.00
0.00
-2.00
-4.00
-6.00
-8.00
Control 1
Control 2
TBI 1
TBI 2
ILF
Percentage Volume Change
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
Control 1
Control 2
TBI 1
TBI 2
SLF
Percentage Volume Change
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
Control 1
Control 2
TBI 1
TBI 2
Thalamus
Percentage Volume Change
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
Control 1
Control 2
TBI 1
TBI 2
Parahippocampal Cortex
Percentage Volume Change
2.00
1.00
0.00
-1.00
-2.00
-3.00
Control 1
Control 2
TBI 1
TBI 2
Parietal Cortex
Percentage Volume Change
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00
-2.50
Control 1
Control 2
TBI 1
TBI 2
Temporal Cortex
Percentage Volume Change
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
Control 1
Control 2
TBI 1
TBI 2

Figure 5: Within the TBI group, there was a significant correlation between poorer Trails A score at time 1 and volume loss in the brainstem and cerebellum during the first interval. Results of this contrast are shown in the glass brain on the bottom left (which shows all significant regions on a transparent map) and in red on the combined figure in the center. There was also a significant correlation between poorer Trails A performance at time 1 and volume loss during the second interval in the same general regions. Results of this contrast are shown in the glass brain on the bottom right and in blue on the combined figure in the center. A False Discovery Rate (FDR) of $p<.05$ utilized (corresponding to a t statistic of greater than 4.271 in the first interval contrast and 3.814 in the second interval contrast), and a cluster threshold was set at 500 contiguous voxels.

Figure 5

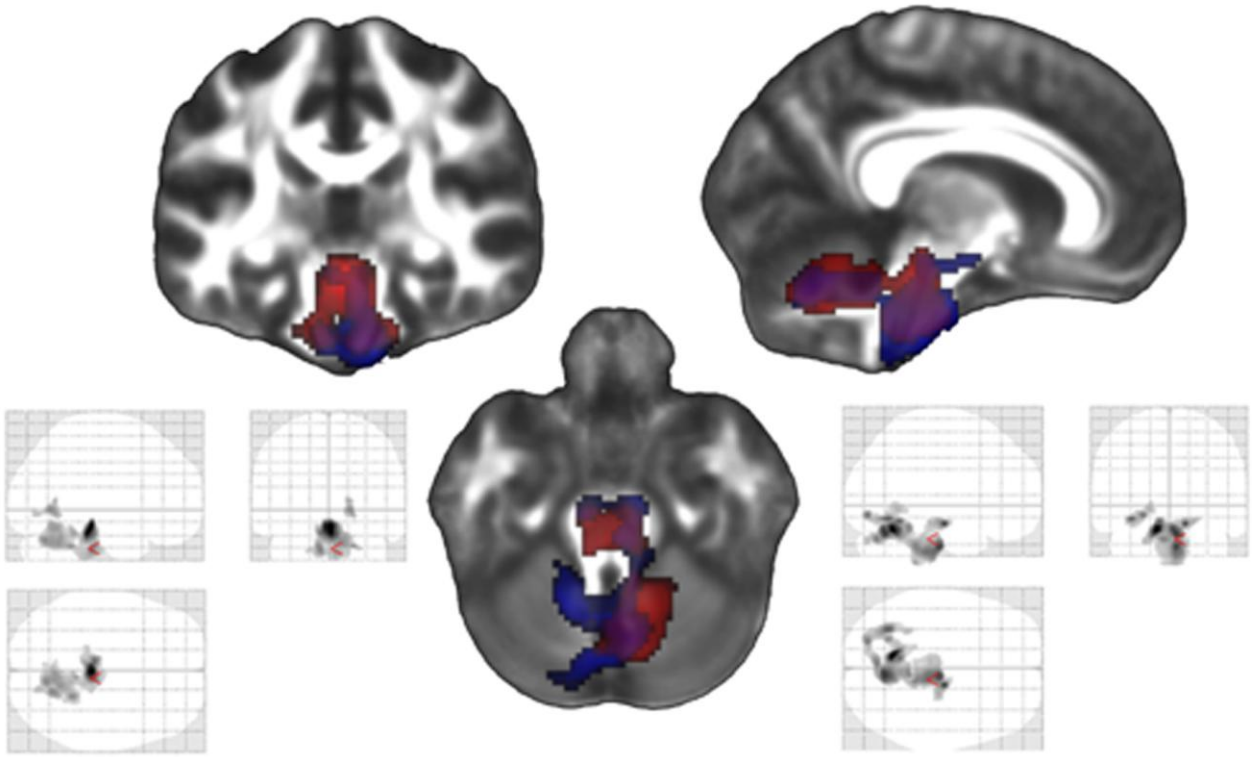
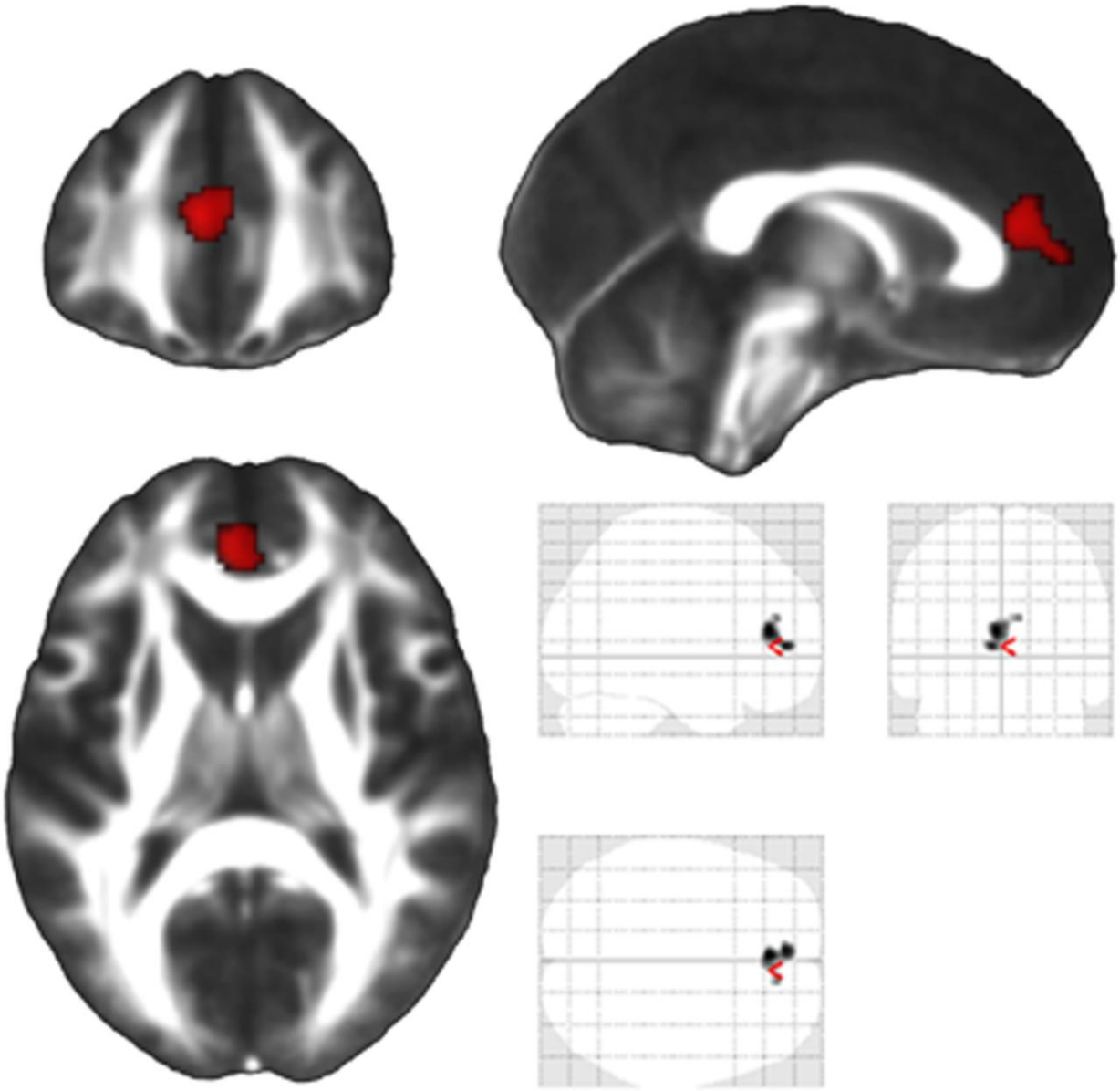


Figure 6: There was a significant correlation between poorer Trails B scores (greater time to completion) at one year post-injury and greater volume loss in the anterior cingulate during the subsequent three year period. The results of this contrast are shown in the glass brain on the bottom right, which includes all significant regions depicted on a transparent background, and in red on the three representative orthogonal sections. A False Discovery Rate (FDR) of $p<.05$ was utilized (corresponding to a t statistic of 3.732), and a cluster threshold was set at 500 contiguous voxels.

Figure 6



Chapter 4:

Individual differences in functional MRI encoding responses after traumatic brain injury: The effect of age, cognitive reserve and injury severity

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Abstract

Traumatic brain injury (TBI) leads to an array of cognitive and functional sequelae from which recovery is generally slow and nearly always incomplete. Episodic memory function (comprising the separable processes of encoding, consolidation and recall) is critical to successful independent living, and lingering memory complaints and measured impairment are common among TBI patients. This blood-oxygen level-dependent (BOLD) functional MRI (fMRI) study tested episodic encoding neural responses in nineteen TBI patients and 51 healthy controls in a four-year longitudinal study. At approximately 2 months post injury, the TBI group mean activation did not differ significantly from controls, but the TBI patients exhibited much greater variability in activation that we sought to explain by examining individual differences in age, cognitive reserve, and injury severity. A significant association was observed where more severe injury correlated with activation in areas outside the normative ventral and mesial temporal lobe structures observed in controls; Lower cognitive reserve was associated with hypo-activation in the ventral temporal lobe compared to controls participants. Longitudinal analyses were conducted to identify factors that influence a return to a normal pattern of activation among the TBI patients: 100% of young adult TBI patients (age 18 to 28) demonstrated normalized task-related activation at the four-year follow-up, while 100% of patients ages 29 and over remained atypical four years post-injury. We also describe select TBI cases in more detail to provide qualitative support for quantitative analyses. Future work determining whether these patterns are upheld in a larger sample is necessary to validate the clinical relevance of this work.

Introduction

Traumatic Brain Injury (TBI) is varied in the circumstances of injury, location, diffuse versus focal nature of the injury, and cognitive and functional outcomes (Bigler & Maxwell, 2011; Cernich et al., 2010; Dikmen et al., 2009). While there are some cognitive deficits and neurological sequelae that are common among TBI patients, many of the specific functional and neurological consequences experienced by individuals are resultant of the localization, severity and cause of specific injuries (Rosenbaum & Lipton, 2012). Memory problems, however, are among the most commonly cited complaints and functional deficits that afflict TBI patients regardless of specific injury characteristics, and are a common cause of disability (Catroppa & Anderson, 2002; Clune-Ryberg et al., 2011; Gale et al., 1993; Vakil, 2005). An interesting scientific problem in the investigation of TBI outcome is the multiple sources of variability. In the domain of memory, some patient's post-injury memory function may be in the normal range, yet others remain chronically impaired. Several factors are known to positively impact recovery potential, including lesser injury severity, heightened cognitive and brain reserve, and youth (Chapman & McKinnon, 2000; Ghosh et al., 2009; Jeon et al., 2008; Kesler et al., 2003; Kiraly & Kiraly, 2007; Levin, 1995; Ponsford et al., 2000; Povlishock & Katz, 2005). It is not clear, however, through what processes these contributions are made. The current study uses fMRI to investigate how some of these factors influence brain function following injury, and assesses whether these factors relate to functional recovery and normalization.

Functional MRI is a powerful tool that can be used to identify neurological processes underlying cognitive functions, and may provide clinically relevant insight about the neural systems that give rise to impairment and recovery. In the case of memory encoding, this technique can be used to identify neural systems involved in encoding, and how cognitive

strategy shifts may lead to altered patterns of functional activation in patient populations. Furthermore, fMRI can be used to assess outcome over time by determining whether or not patients with atypical patterns of activation close to the time of injury return to normalized activation patterns over time (Laatsch & Krisky, 2006; Sanchez-Carrion, Fernandez-Espejo et al., 2008). This technique has been cited as a useful tool in both identifying damage and tracing recovery (Hunter et al.; Kou et al.).

Research assessing functional activation on a variety of cognitive tasks indicates that TBI patients demonstrate much broader variability in activation patterns compared to controls, and studies have reported group mean activations that are reduced (Mani et al., 2007; Sanchez-Carrion, Gomez et al., 2008), increased (Scheibel et al., 2009), or differently localized (Strangman et al., 2008) than activation patterns observed in healthy controls. This variability likely reflects the individual differences at the patient level in this disorder; however, factors determining whether patients will demonstrate increased, decreased, or differently localized activation patterns have not been well characterized. Previous research has demonstrated slight increases in activation to an encoding task in TBI patients compared to healthy controls (Arenth et al., 2012). However, still little is known about the neural correlates of encoding in this patient population, nor has previous work investigated how differences in encoding activation change or persist over time, or whether these differences relate to measures of injury severity, cognitive reserve or general recovery.

The current study sought to investigate memory encoding systems following TBI, and identify factors and individual differences that may account for variability in encoding systems. To address both the commonalities and heterogeneity of our TBI patients, the current report includes group-wise analyses based on a priori hypotheses, analyses utilizing post-hoc

categorization of patients into sub-groups demonstrating particular functional responses, and individual case reports to provide qualitative support for quantitative findings.

We assessed nineteen TBI patients at two months post-injury and again four years post-injury to determine the extent to which functional activation during memory encoding (as assessed by fMRI) differed between patients and fifty-one healthy controls at baseline, and whether any observed differences persisted over time. The control group was used to determine a normal pattern of task-related activation, and was large enough to be statistically robust to the influence of outliers (Desmond & Glover, 2002). The task employed was intentionally designed to be very simple so that even patients with memory impairment would be able to perform at a high level (Johnson et al., 2006). We hypothesized that the TBI group would exhibit greater variability in task-related activation compared to control participants and that some of this variability would be accounted for by age, an estimate of cognitive reserve, and an estimate of injury severity. We assessed whether individual differences in patient activation patterns could be explained by injury severity, general memory function, or cognitive reserve and predicted that all three of these factors would be at least somewhat explanatory (Sanchez-Carrion, Fernandez-Espejo et al., 2008; Scheibel et al., 2009). We also hypothesized that TBI patients demonstrating atypical activation patterns at the initial scan would display more normalized activation at follow-up.

Methods

TBI patients

Forty-seven TBI patients participated in an initial fMRI scan, and 21 returned for a four year follow-up visit. Useable fMRI data was acquired in 19 participants at both visits (differences in acquisition necessitated the exclusion of 2 scans). The mean age of the final group was 35.6 ± 11.9 years at the beginning of the study; mean education was 13.1 ± 1.7 years. The final TBI group consisted of 3 women and 16 men. The majority of the patients included in the study received acute treatment at the University of Wisconsin Hospital and Clinics level 1 trauma center and were referred from the departments of Neurosurgery, Trauma and/or Rehabilitation. The inclusion criteria for TBI consisted of involvement in a rapid impact injury to the brain (such as a motor vehicle accident or fall) causing a loss of consciousness, and subsequent admission for emergency medical attention. Patients sustaining penetrating or open head injuries were excluded from the study. Depressed skull fracture was not excluded (one patient). The average GCS score of patients at 24 hours post-injury was 10.6. For a detailed overview of clinical characteristics and injury descriptions, see Table 1. All TBI patients were less than 3 months post-injury at their first visit, and most were studied between 8 and 12 weeks post-injury, depending on their availability and other medical issues related to their injury. Exclusion criteria consisted of current major Axis I psychiatric disease or history of non-injury related major medical conditions (cancer, diabetes, or previously diagnosed neurological condition). All patients gave informed written consent under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board.

Healthy Controls

53 control participants were recruited from the community and from the University of Wisconsin campus via advertisement. Useable fMRI data was acquired in 51 participants (differences in acquisition necessitated the exclusion of 2 scans). The mean age of the control group was 27.0 ± 8.4 years; mean education was 15.1 ± 2.2 years; and there were 27 men and 24 women. Exclusion criteria were identical to the TBI group, with the exception that head injury was also exclusionary for controls. All participants gave informed written consent under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board.

Study Procedures

TBI patients participated in two testing sessions, each consisting of neuropsychological testing and fMRI. TBI patients were tested at two visits. Visit 1 was acquired approximately 2.5 months post injury (m= 73.4 days post injury, ranging from 30 days to 139 days). Visit 2 was acquired approximately four years post injury (m= 1589 days post-injury, ranging from 1358 days to 2015 days). Controls were utilized to identify the normative pattern of activation to an fMRI task. While some controls had more than one visit, only the baseline data collected from control participants were employed in this study to characterize the normal pattern of brain activity.

Neuropsychological Examination

Participants received a neuropsychological battery at each visit. Tasks included COWAT (Controlled Oral Word Association Test) (Lezak, 1995), WRAT-III (Wide Range Achievement Test) Reading Subtest (an approximation of pre-injury intellectual attainment) (Wilkinson & Jastak Assessment systems., 1993), CVLT-II (California Verbal Learning Test-II; PsychCorp Pearson Assessments), BVMT-R (Brief Visuospatial Memory Test-Revised; PAR Inc.), Finger Tapping (Spreen & Strauss, 1991), WAIS-III Digit Span (Wechsler Adult Intelligence Scale

third edition; PsychCorp Pearson Assessments), and Trail Making Tests A and B (Reitan, 1955) was administered to each participant. Participants were also administered several questionnaires, including a health history questionnaire, the BDI-II (Beck Depression Inventory), the STAI (State-Trait Anxiety Inventory) and the MPAI (Mayo-Portland Adaptability Index, given only to TBI patients at the follow-up visit). For a summary of task performance and questionnaire results, see Table 2. A composite variable representing Cognitive Reserve for TBI patients was calculated as follows: z(WRAT-III reading subtest score) + z(years of education) + -z(age at injury). Another composite variable representing Memory Function was calculated by adding patients' T-scores on the BVMT-R and CVLT-II at the initial visit.

fMRI Task

The task consisted of serial presentation of novel (NV) and previously viewed (PV) line drawings obtained from a published set (Snodgrass & Vanderwart, 1980). PV items were learned during two training sessions, 45 minutes and 15 minutes prior to the fMRI task. The training set consisted of five items with similar image complexity to novel items. The training set was presented 15 times during each of the two training sessions for a total of 30 exposures to each item. The order of item presentation was pseudorandomized within each set presentation. Each item was presented for 3000 ms with no gaps between stimuli. Participants were instructed to try to remember the small set of training items. Because we were specifically interested in memory encoding, the training session was intended to desensitize subjects to the training set. The training session creates a situation where stimulus specific neural adaptation occurs such that regions involved in processing and encoding the information, including the hippocampus, no longer respond to the training items during the scanning session (Johnson et al., 2004).

During the fMRI scan, PV items and NV items were intermixed in a variable length block format as previously described (Johnson et al., 2006; Trivedi et al., 2008). A picture was presented every three seconds for the duration of the scan together with on-screen instructional text. The cognitive set was the same throughout the task, which was a decision about whether or not the current item was previously viewed ('old') or novel ('new'). Participants selected their responses using a two-button device held in the right hand. The first finger was used to identify PV items and the middle finger was used for NV items. All stimuli were presented for 2800 ms with a 200ms inter-stimulus interval. There were no fixation periods or null events. Items within each condition occurred as trains of events and ranged from a single item to five consecutive items. This variability in epoch length was implemented to reduce condition predictability while preserving the comparatively greater statistical power and shorter duration of boxcar style paradigms (Liu et al., 2001). Two iterations of the task were sequentially presented using the same PV items but different NV items, and the order of the two iterations was counterbalanced across subjects. The total task duration was 9 min 24 s.

Scanning Procedures and Image Processing

Scanning was done using a General Electric 3.0 T scanner outfitted with an MR-compatible button-box and high-resolution goggle system (Avotec Products). The head was constrained by foam padding. The software Presentation was used to deliver the visual stimuli, via the goggle system, and to record responses. A T2* gradient, echo-planar imaging (EPI) pulse sequence was used. Higher order shimming was applied to the static magnetic field (B0). The EPI parameters were: echo time= 30ms; repetition time (TR)= 2000ms; flip angle= 90º; acquisition matrix= 64x64 voxels; field of view= 240mm. Thirty sagittal slices were acquired within each TR. Voxel resolution was 3.75mm x 3.75mm x 5mm (4mm thick slices with 1mm

skip). A time course of 141 temporal volume images was collected, of which the initial 3 image volumes of each scan were discarded. Following the functional scans and field mapping, a T1 weighted inversion recovery prepped volume and T2 weighted anatomic images were acquired and later viewed by a radiologist for abnormalities that were inconsistent with the diagnosis.

The 4D time series was motion-corrected using SPM8. The field map from each subject was then applied to the time series. This was followed by spatial normalization into a standard atlas space (using the T2* weighted template provided with SPM8), and spatial smoothing with an 8mm Gaussian kernel. To estimate single-subject activations, the analysis employed the canonical hemodynamic response function, high-frequency signal filtering (high pass filter= 128 s) and estimated temporal autocorrelation. The contrast NV>PV was computed for each subject in SPM8.

Statistical Analysis

Statistical analyses of fMRI data were carried out in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). A one-sample t-test of the control group was used to determine regions normally active during the task. A one-sample t-test of the TBI group was also completed for visual comparison. Two-sample t-tests were used to identify group-wise differences in the pattern of task-related activation. Age, gender, and education were used as covariates in analyses comparing patients and controls. Paired-samples t-tests were used to identify regions in which the TBI group demonstrated differences in the group-wise pattern of activation between the initial and follow-up scans. Correlations between task-related activation and other measures (GCS, MPAI, BVMT-R/CVLT-II, cognitive reserve) were calculated using linear regression models in SPM8, where non-fMRI measures were independent variables and fMRI maps were dependent variables. All t-tests and regressions carried out on fMRI data were

conducted at p=.001 (uncorrected) and a cluster threshold of 50 voxels. Correlations and statistical tests not involving fMRI data were carried out in SPSS19.

Based on their extent of activation, TBI patients were divided post-hoc into hyper-activators, normal-activators and hypo-activators. The procedure for dividing participants was based on deviation from the mean and was carried out as follows: A mask of the control group NV>PV one-sample t-test result map, calculated at the low threshold of p=.01, was applied to each TBI patient's NV>PV result map. A participant was considered a normal-activator if he or she had clusters of at least 400 contiguous voxels in each of the bilateral ventral and mesial temporal lobes within the region covered by the control mask, and had no clusters of more than 300 voxels in regions outside the mask that were superior to the corpus callosum or anterior to the midline. A participant not demonstrating clusters of at least 400 voxels in the bilateral inferior temporal lobe (ITL) within the region covered by the control mask was classified as a hypo-activator. A participant demonstrating clusters of at least 400 voxels in the bilateral ITL within the region covered by the control mask and also demonstrating one or more clusters of more than 300 contiguous voxels in regions outside the control mask forward of the midline or superior to the corpus callosum was classified as a hyper-activator. These cut-offs were derived based on the following: the average cluster size of bi-lateral ventral mesial temporal lobe clusters in control participants was 1623 voxels on the left and 1518 voxels on the right. The standard deviations of these clusters among control participants were 1216 on the left and 1115 on the right. Therefore, a patient not having clusters of at least 400 voxels on each side would be more than one standard deviation below the control mean. In control participants the mean size of the largest cluster outside the canonical pattern, forward of the midline and superior to the corpus callosum was 174 voxels with a standard deviation of 123 voxels. Therefore, a cluster in the

specified extra-normative regions that exceeded 300 voxels in size would be more than one standard deviation larger than the control mean for maximum extra-normative cluster size.

Results

Demographic and Behavioral Results

Group demographics are shown in Table 1. Controls were significantly younger than TBI patients (t=2.601; df=68; two-tailed p=.01) and had significantly higher levels of education (t=3.528; df=68; two-tailed p=.0008). In addition, there was a significantly lower proportion of females in the TBI group compared to the control group ($\chi 2(1,69)=5.713$, p=.02). Patient injury characteristics are also shown in Table 1. TBI patients performed significantly worse than controls on the DSPAN, Trails A, Trails B and COWAT tests at the initial visit. At follow-up, TBI patients had improved to normal levels on the Trails A, Trails B and COWAT tests, but remained impaired on the DSPAN test. TBI patients did not differ significantly from controls on measures of pre-morbid intelligence (WRAT-III Reading Subtest), nor did Memory Function differ between TBI patients and control participants. TBI patients had higher rates of depression (BDI-II) but not anxiety, than the control participants (STAI-Trait and STAI-State). Neuropsychological test performance summaries are shown in Table 2.

Behavioral Performance

Both patients and controls performed the fMRI task at a high level, and as expected an independent-samples two-tailed t-test demonstrated that there was not a significant difference in performance between the two groups. Controls attained 98.7% (SD=2.3%) accuracy, patients attained 93.7% (SD=13.0%) accuracy (df=68; p=.22). At follow-up the patient group attained 97.6% (SD=4.5%) accuracy. A paired-samples two-tailed t-test did not indicate a significant difference in patient accuracy between the initial scan and follow-up (df=18; p=.24). The patient group had a mean reaction time of 900 ms with a standard deviation of 177 ms at the initial scan and a mean reaction time of 787 ms with a standard deviation of 161 ms at the follow-up scan.

The control group had a mean reaction time of 830 ms and a standard deviation of 135 ms. Two-tailed independent-samples t-tests indicated that the patient group reaction time was significantly different from the control group reaction time at the initial visit (df=68; p=.02) but not at follow-up (df=68; p=.13). A two-tailed paired-samples t-test indicated a significant difference in the mean patient reaction time between initial scan and follow-up (df=18; p=.00009).

Imaging Results

To show the effect of task, results of a one-sample t-test on the control data are shown in Figure 1. The control group demonstrated substantial activation in the bi-lateral ventral temporal lobes as well as in a cluster in the left frontal cortex. This is consistent with previous studies that have used this task in healthy populations. A one-sample t-test of the TBI patients revealed a similar pattern of activation (Figure 2). Two-sample t-tests indicated that there were no significant differences in the pattern of activation between initial patient scans and control scans, nor were there significant differences between follow-up scans and control scans. Paired-samples t-tests within the patient group demonstrated that there were no regions in which TBI patients demonstrated differences in the overall group-wise pattern of activation between the initial and follow-up scans. However, visual examination of individual patient and control activation maps suggested that there was considerably greater variability among TBI patients compared to controls.

Four primary measures were tested for correlation with fMRI activity at the initial scan: GCS at 24 hours post-injury, Cognitive Reserve, Memory Function and MPAI. Correlations between activation and Cognitive Reserve and Memory Function were also tested in controls. Regression analyses revealed that higher GCS scores (less severe injuries) correlated with greater task-related activation in the bilateral middle temporal gyrus as well as in regions of the right

frontal and right ventral and mesial temporal cortices (Figure 3). Higher Cognitive Reserve correlated with greater activation in a small region of the right ventral and mesial temporal lobe among TBI patients but not among controls (Figure 3). There were no significant correlations between fMRI activation and Memory Function in either group. Among patients there was a significant correlation between fMRI activation at the initial scan and MPAI at follow-up in the right insular cortex and right parahippocampal gyrus (Figure 3).

Variation in Activation among the TBI Group

Within the TBI patient group, eight participants (42%) were hypo-activators at the initial scan, seven (37%) were hyper-activators and four (21%) were normal-activators. Within the control group, five (10%) participants were hyper-activators, eight (16%) were hypo-activators and thirty-eight (74%) were normal activators. The proportion of atypical activators in the TBI group was significantly greater than the proportion of atypical activators in the control group (TBI: 79% atypical; Control: 26% atypical; $\chi 2(1,69)=16.483$, $p=.0001$). While this statistic is merely descriptive due to the fact that criteria for normal-activator status were derived based on control group data, it is nonetheless useful. It demonstrates that while there were no significant differences in group-wise patterns of activation, the normal mean activity exhibited by TBI patients is due to the counter-balancing effect of opposing atypical activation patterns in subsets of patients.

Independent-samples two-tailed t-tests were used to investigate differences between groups of patients with different activation profiles. The following measures were assessed: GCS24, MPAI, Cognitive Reserve, Memory Function, and WRAT-III reading subtest (an approximation of pre-morbid intelligence). Results demonstrated that hyper-activators had a significantly lower GCS24 score than normal activators ($p=.004$), and hypo-activators had

significantly lower Cognitive Reserve than either normal-activators (p=.03) or hyper-activators (p=.008). There were no significant differences in task performance between normal-, hyper- and hypo-activators. For a complete report of the results of tests comparing patients with different activation profiles, see Table 3.

Longitudinal Outcomes

Of the fifteen TBI patients demonstrating atypical patterns of task-related activation at the initial scan, six demonstrated a normal activation pattern at the four-year follow-up scan. Spurred by previous research indicating that younger patients exhibit greater levels of recovery following TBI, we assessed whether age could be used to help explain which patients demonstrated a normal activation pattern at follow-up and which patients remained atypical. An independent-samples two-tailed t-test demonstrated that those recovering normal activation were significantly younger than those remaining atypical (p=.0001). Furthermore, we observed a dichotomized split wherein all patients demonstrating a normal activation pattern at follow-up were ages 28 and younger and all remaining patients demonstrating atypical activation patterns at follow-up were ages 29 and older (Figure 4). Of those patients that remained atypical, seven had the same activation status at the initial and follow-up scans, one hyper-activator became an hypo-activator, and one hypo-activator became a hyper-activator. Patients returning to normal activation status did not differ from those remaining atypical in terms of GCS24, WRAT-III reading subtest performance, Memory Function or MPAI.

Case Studies

Due to the qualitative nature of this analysis, four individual patient cases are presented in detail to demonstrate how these analyses may apply to individual cases. Patient 1 is a 23 year old male who sustained a TBI in a motor vehicle accident. The vehicle rolled over and the

patient, who was unrestrained, was ejected. This patient had a GCS upon admission of 7 and at 24 hours post-admission his GCS remained unchanged (GCS24=7). This indicates that his injury was moderate to severe (mean patient GCS24 =10.63). He had multiple large hemorrhagic contusions in both the left and right frontal lobes, a fracture to his right occipital bone and small subdural hematomas adjacent to the right temporal lobe. His Cognitive Reserve was 1.60, putting him above the patient average. (Cognitive Reserve Composite score is the sum of three z-scores, and has a mean of 0 and SD of 1.63.) This patient's WRAT-III reading subtest score of 112 was above average (patient m= 105.8, control m= 104.8), however his Memory Function score of 59 was well below average (patient m= 92.3, control m= 96.5). This patient's GCS score puts him in the moderate to severe injury category, and during fMRI he presented as a hyper-activator at baseline (Figure 5). He demonstrated a cluster of 2907 voxels in the left ventral and mesial temporal lobe and 2173 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 786 voxels in the central interhemispheric cortex. However, by the four year follow-up he demonstrated a normal activation pattern (Figure 5). He demonstrated a cluster of 2167 voxels in the left ventral and mesial temporal lobe and 1598 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of just 60 voxels. This return to normal activation occurs despite the fact that this patient's MPAI score of 11 indicates poor perceived recovery (m=21.9).

Patient 2 is a 31 year old female who sustained her TBI in a motorcycle accident. She was not wearing a helmet. She had a GCS at admission of 3 and a GCS24 of 10, indicating a moderate to severe injury. She had a hemorrhagic contusion to her right frontal lobe, subarachnoid bleeding in the left fronto-temporal region, and subdural hematomas in the inter-hemispheric fissure and left parietal convexity. She had a Cognitive Reserve score of 1.47, which

was above average. Her WRAT-III reading subtest score of 107 was close to average and her Memory Function score of 112 was classified as high. Like Patient 1, Patient 2 hyper-activated to the task at the initial scan (Figure 6). She demonstrated a cluster of 879 voxels in the left ventral and mesial temporal lobe and 1024 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 2086 voxels in the left parietal lobe. Unlike Patient 1, however, Patient 2 is over 30 years of age. Patient 2 remained an atypical-activator at the four year follow-up scan, despite her better perceived outcome (MPAI=31) (Figure 6). At follow-up she demonstrated a cluster of 3622 voxels in the left ventral and mesial temporal lobe and 1884 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 4326 voxels in the left parietal lobe.

Patient 3 is a 25 year old male who sustained his TBI while "car-surfing"; the patient fell from the roof of a moving vehicle on which he was standing. He was initially unconscious for about 30 minutes post-injury. He had a GCS24 of 15, indicating a mild injury. He had a right parietal epidural hematoma, a partieto-occipital subdural hematoma, right fronto-temporal contusions and multiple fractures to his right temporal bone. He has a below average Cognitive Reserve score of -1.25. His WRAT-III reading subtest score of 97 and Memory Function score of 97 were close to average. Patient 3 has a relatively high GCS24 score, but a relatively low level of Cognitive Reserve. At his initial visit, he hypo-activated to the fMRI task (Figure 7). He demonstrated a cluster of 208 voxels in the left ventral and mesial temporal lobe and 164 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 54 voxels. At the follow-up scan, this patient demonstrated a normative pattern of activation (Figure 7). He demonstrated a cluster of 1297 voxels in the left ventral and mesial temporal lobe and

2305 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 110 voxels. Patient 3 had an MPAI score of 8.

Patient 4 is a 49 year old male who sustained his injury in a motorcycle accident. He was not wearing a helmet. While the patient reported brief unconsciousness following injury, both his GCS at admission and GCS24 were 15. Neurologically this patient exhibited shearing of the corpus callosum, diffuse axonal injury (DAI), and subdural hematoma of the left parietal lobe. His Cognitive Reserve score is -2.44. WRAT-III reading subtest score (107), Memory Function score (105) and MPAI (20) were all close to average. At the initial scan, Patient 4 presented as a hypo-activator. He exhibited no significant voxels in the left ventral and mesial temporal lobe and 44 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 43 voxels. At follow-up, Patient 4 remained atypical in his task-related activation. He demonstrated a cluster of 180 voxels in the left ventral and mesial temporal lobe and 220 voxels in the right ventral and mesial temporal lobe, and a maximum extra-normative cluster of 70 voxels. (Figure 8).

Discussion

There were no group-wise differences in the pattern of activation demonstrated by TBI patients compared to controls. There was, however, significantly greater variability among TBI patients compared to controls, with subjects demonstrating both hyper- and hypo-activation. This is consistent with previous fMRI research in this patient population (Mani et al., 2007; Sanchez-Carrion, Gomez et al., 2008; Scheibel et al., 2009; Strangman et al., 2008). This is likely due both to damage, which could preclude the use of affected brain regions in fMRI tasks, and to plasticity, which could involve the recruitment of regions not normally used to complete the task. Correlational fMRI analyses indicated that Cognitive Reserve, Memory Function and injury severity were all relevant to activation patterns. Higher Cognitive Reserve was associated with greater activation in bilateral regions of the ventral and mesial temporal lobes, a region normally active during the task. Poorer general memory function outside the scanner was associated with greater activation both within and outside canonical regions of activation. This could be because patients with poorer general memory capabilities found the task more difficult, and were thus more engaged during scanning. Less severe injuries were associated with greater activation in the middle temporal gyrus, a region that was active among controls participants.

Our investigation of why some patients hypo-activated and others hyper-activated elucidated two primary trends: those with lower GCS scores were more likely to hyper-activate, while those with lower cognitive reserve score were more likely to hypo-activate. More severe injuries likely required greater recruitment of outside regions, resulting in hyper-activation. It is also possible that there was a derangement of neurovascular coupling, or an increase in blood flow with inadequate metabolism of oxygen due to injury, either of which could result in increased BOLD signal without increased neural activity. Lower cognitive reserve, on the other

hand, may have prevented the full engagement of necessary structures following injury. Lower cognitive reserve could also be associated with reduced synaptic complexity or fewer redundant neural systems, which could reduce the observed BOLD signal. Future work should investigate whether these patterns of hyper- and hypo-activation are maintained in a larger sample, and attempt to determine which effect (low GCS or low Cognitive Reserve) is most likely to dominate in a patient having both of these qualities.

Both hyper- and hypo-activation could also reflect use of different cognitive strategies by patients needing to circumvent the use of damaged brain regions. This is probable considering that performance on the memory encoding task inside the scanner did not significantly differ from controls, while reaction times did. A previous study (Arenth et al., 2012) identified clusters of increased activation to a verbal memory encoding task, which the authors interpreted as evidence of TBI patients utilizing a different (and less effective) cognitive strategy from controls. That study focused on differences between patients and controls on a verbal memory task using fewer subjects (12 patients, 12 controls) compared to the current study, and no within-run control was employed. The current study used a simpler encoding task but nonetheless observed hyper-activation in many subjects. More research is necessary to determine whether differing cognitive strategies do indeed underlie this increased BOLD signal.

In addition to determining why patients demonstrated different atypical activation patterns, we also sought to investigate why some of the patients demonstrated a normalized pattern of activation at follow-up while others did not. Initially, we looked at GCS, Cognitive Reserve and Memory Function as potential predictors. We determined the Cognitive Reserve held predictive value. Breaking this measure down into its component parts, however, revealed that age was more closely associated with a return to normal activation status than either of the

other two components of the Cognitive Reserve score, or the composite. In this small set of subjects we observed a dichotomy in which all TBI patients ages 25 and under initially demonstrating atypical activation pattern returned to a normal pattern of activation at the four year follow-up, while none of the initially atypical activators over the age of 30 demonstrated such normalization. (There were two patients between the ages of 25 and 30, both of whom exhibited normal activation patterns at the initial scan. The 28 year old patient remained normal at follow-up while the 29 year old patient exhibited hyper-activation at follow-up.)

While it is not clear why younger patients demonstrated normalized patterns of BOLD activation while older patients did not, it should be noted that the two groups did not demonstrate differing levels of recovery in terms of neuropsychological task performance or MPAI scores. Therefore, it is possible that the younger patients experienced neural reorganization that facilitated BOLD normalization, while older patients adopted new cognitive strategies to achieve some degree of recovery without functional normalization. Several studies have indicated that advancing age is associated with poorer prognosis following TBI (Jeon et al., 2008; Marquez de la Plata et al., 2008; Ponsford et al., 2000). It is nonetheless surprising to observe an effect of age in a relatively young patient group, and this effect merits further research. It should also be noted that previous research on this cohort of TBI patients has demonstrated that both pervasive volume loss (concentrated in white matter) and changes in white matter integrity (with both improvements and decrements occurring in subsets of brain regions) are co-occurring with the functional changes observed in the current study (Farbota et al., 2012a; Farbota et al., 2012b). These changes may contribute to brain adaptations underlying functional differences.

Limitations

Some limitations of this study deserve mention. One limitation is that age, gender composition and education level differed between the two groups, possibly affecting results. The non-geriatric TBI population is generally younger, more heavily male and less educated than the non-geriatric general population. Previous studies of volumetric change in TBI patients that use age, gender and/or education matched controls (Kim et al., 2009), and those that use controls that are not thusly matched (Bendlin et al., 2008) return similar results. Furthermore, we included age, gender and education as covariates in our statistical models to decrease the effect of these differences.

Additionally, the results of this study may be limited by the small size of the final TBI group. The TBI population is itinerant, and therefore difficult to track for long periods of time. While statistical analyses indicated there were not significant differences in age, education or injury severity within the TBI group between those who completed only the first visit and those who returned for four-year follow-up, the high attrition could still have biased our results. Finally, it should be noted that the memory encoding task used in this study only assesses one very specific function, and it is therefore important to be cautious in generalizing these results to other cognitive tasks.

Conclusion

The results of this study indicate that variation in the pattern of activation to a memory encoding task can be partially explained by differences in cognitive reserve and injury severity. Specifically, patients demonstrating lower cognitive reserve are more likely to demonstrate hypo-activation while those with more severe injuries are more likely to demonstrate hyper-

activation. Furthermore, this study indicates that age may be a critical factor in determining whether or not activation patterns normalize long term.

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Table 1: *Individual level subject demographics for patients and controls and select patient injury characteristics*

Patients (n=19)

Subject	Age	Education	Sex	GCSad	GCS24	Hrs15	Scan1	Injury Notes
1	19	12	M	3T	7	334	139	DAI
2	37	16	M	14	15	1	97	DAI , subarachnoid hemorrhage
3	18	11	M	11	11	76	69	Contusions, subarachnoid hemorrhage
4	23	12	M	7	7	541	59	Contusions, skull fracture, subdural hematomas
5	31	16	F	3T	10	444	84	Contusions, subarachnoid bleeding, subdural hematoma
6	49	12	M	15	15	0	91	DAI, callosal shearing, subdural hematoma
7	19	14	F	3T	11	91	90	Subarachnoid hemorrhage, subdural hematoma
8	24	14	M	3	7	662	69	DAI, contusions, epidural hematoma, subarachnoid hemorrhage
9	48	12	M	14	15	4	94	Subdural hematoma
10	39	10	M	3T	6	835	92	Extensive contusions, subdural hematoma
11	25	12	M	15	15	0	60	Multiple skull fractures, subdural hematoma, contusions
12	49	12	M	3T	8	110	25	Epidural hematoma, subarachnoid hemorrhage
13	45	12	M	3T	14	270	30	Skull fracture, frontal contusion
14	29	13	M	3	14	179	56	DAI, contusions, skull fracture
15	52	16	F	11	13	97	55	Skull fracture, subarachnoid hemorrhage, subdural hematoma,
16	51	12	M	7	7	726	79	Depressed skull fracture, subdural and epidural hematomas
17	34	12	M	15	15	0	61	Epidural and subarachnoid hematomas
18	19	13	M	3T	5	168	70	Extensive cortical and callosal shearing, multiple hemorrhages
19	37	14	M	9	7	116	74	DAI, subarachnoid hemorrhage, subdural hematoma, shearing
Mean (SD)	35.4 (12.0)	12.9 (1.8)	82% M	7.2 (5.0)	10.2 (3.5)	276.7 (269.7)	75.5 (28.2)	

Controls (n=51)

	Age	Education	Sex
Mean (SD)	26.9 (8.4)	15.1 (2.2)	52% M
p	0.0112*	.0008*	.0168*

Note: Age of participants indicates the age of participants at the start of the study. P-values are based on the results of two-tailed independent-samples t-tests for age and education and a chi-squares test for gender proportions. Abbreviations are as follows: GCSad= Glasgow Coma Scale score at hospital admission, GCS24= Glasgow Coma Scale score 24 hours post-injury, Hrs15= Number of hours before patient reached a GCS score of 15, Scan1= Number of days between injury and initial scanning, DAI= Diffuse axonal injury, T= Patient was intubated at the time of GCS assessment.

Table 2: *Neuropsychological test performance*

	Controls n, **Mean** (SD)	**TBIs Initial Visit** n, **Mean** (SD)	**TBIs Follow-Up** n, **Mean** (SD)
WRAT-III (Reading)	51, **104.8** (10.8)	19, **105.8** (8.7)	14, **104.2** (7.6)
DSPAN	48, **18.4** (3.6)	18, **15.6** (3.9)**	14, **16.0** (4.3)*
TRAILS A (seconds)	40, **27.6** (9.1)	18, **37.0** (19.0)*	15, **30.5** (12.1)†
TRAILS B (seconds)	40, **59.4** (20.7)	17, **89.5** (50.0)***	15, **74.0** (49.8)†
COWAT	40, **39.6** (12.6)	18, **29.7** (10.4)**	15, **36.8** (11.1)†
FT DOM	26, **49.2** (8.2)	17, **47.9** (12.3)	15, **46.4** (6.2)
BVMT-R	50, **47.8** (13.3)	19, **48.4** (12.6)	17, **47.9** (12.9)
CVLT-II	50, **48.7** (13.6)	19, **47.4** (11.2)	18, **47.7** (12.0)
BDI-II	27, **4.8** (5.2)	19, **9.7** (6.3)**	19, **8.4** (6.6)*
STAI-T	42, **29.0** (6.9)	19, **30.2** (7.5)	17, **29.1** (5.8)
STAI-S	44, **30.7** (6.9)	19, **32.4** (6.9)	18, **30.2** (7.5)

Note: Means are of raw scores. Between-groups differences were calculated using independent samples two-tailed t-tests, and significance levels are *p<.05, **p<.01, ***p<.001. Between groups differences are noted by the patient average scores only. Within-groups differences for TBI patients were calculated using paired samples two-tailed t-tests and a significance level of p<.05. Significant differences are denoted by †. Because there were missing values the sample sizes in the paired tests varied. Test abbreviations are as follows: WRAT-III (Reading)= Wide Range Achievement Test III, Reading Subtest-standardized score, DSPAN= Digit Span Test from the Weschler Adult Intelligence Scale, Third Edition, Trails A= Trail Making Test A, Trails B= Trail Making Test B, COWAT= Cognitive Oral Word Association Test, FT DOM= Dominant Hand Finger Tapping Test, BVMT-R= Brief Visuospatial Memory Test, CVLT-II= California Verbal Learning Test, BDI-II= Beck Depression Inventory, STAI-S= State-Trait Anxiety Test state score, STAI-T= State-Trait Anxiety Test trait score.

Table 3: *TBI Patient Characteristics by fMRI Activation Profile at Initial Scan*

	Hyper-Activators n=7 **Mean** (SD)	**Hypo-Activators** n=8 **Mean** (SD)	**Normal-Activators** n=4 **Mean** (SD)
GCS at 24 hours	**8.0** (2.6)*	**11.4** (3.9)	**13.8** (1.9)
Memory Function	**105.1** (20.8)	**96.4** (13.5)	**86.0** (18.6)
Cognitive Reserve	**0.5** (1.1)†	**-1.3** (1.1)*	**1.7** (1.0)
MPAI	**22.1** (13.3)	**25.8** (21.8)	**15.8** (12.9)

Note: Memory Function is the sum of the Brief Visuospatial Memory Test (BVMT-R) and California Verbal Learning Test (CVLT-II) scores. Cognitive Reserve is the sum of z-score of the Wide Range Achievement Test (WRAT-III) Reading subtest score, the z-score of patient education (in years) and the negative z-score of patient age (in years). MPAI= Mayo-Portland Adaptability Inventory. Differences were assessed using independent samples two tailed t-tests. The * symbol indicates that the average value for the hyper- or hypo-activator group is significantly different from the average value for the normal-activator group at $p < .05$. The † symbol indicates a significant difference between the hyper- and hypo-activator groups at $p < .05$.

Figures

Figure 1: A one-sample t-test of control participants (n=51) demonstrated significant activation in the bilateral ventral and mesial temporal lobes, as well as in a region of the right frontal lobe. A p-value of .001 (uncorrected) and a cluster threshold of 50 voxels were employed. Age, gender and education were included as covariates.

Figure 1

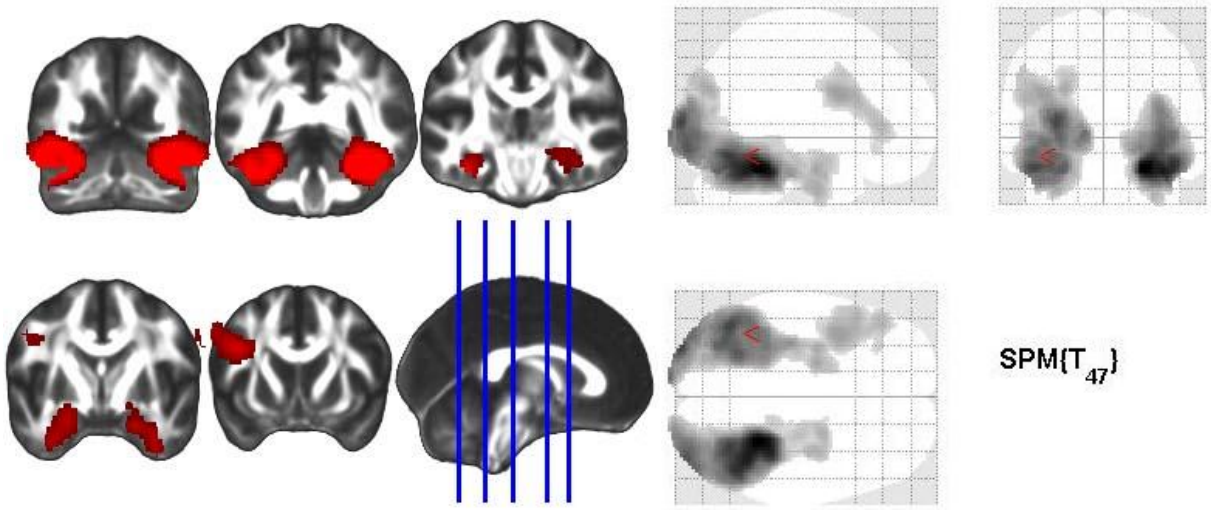


Figure 2: A one-sample t-test of TBI patients (n=19) demonstrated significant activation in the bilateral ventral and mesial temporal lobes, as well as in a region of the right frontal lobe. A p-value of .001 (uncorrected) and a cluster threshold of 50 voxels were employed. Age, gender and education were included as covariates.

Figure 2

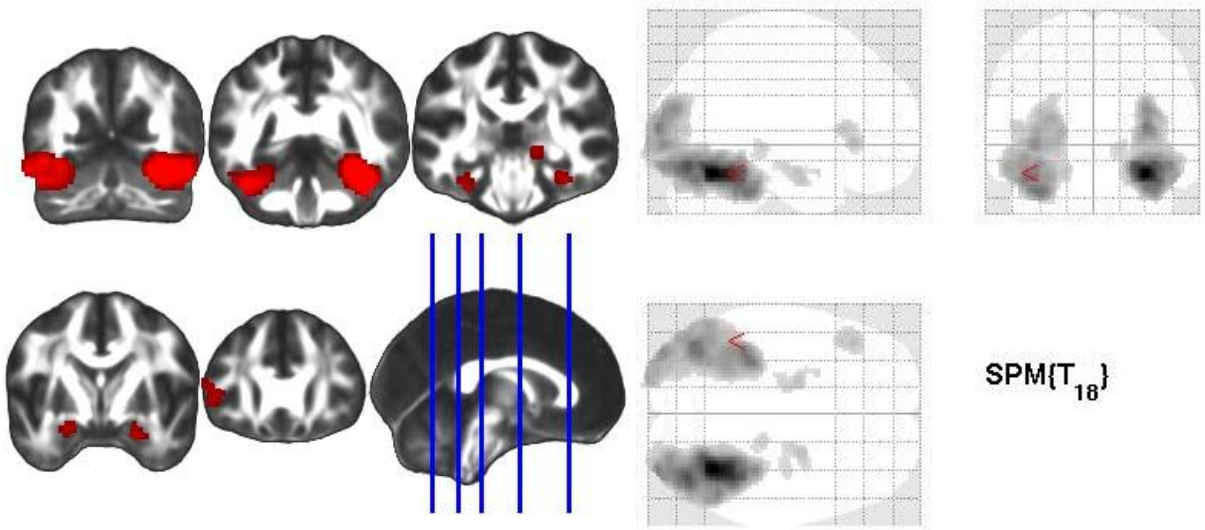


Figure 3: Linear regressions in which neuropsychological variables (24-hour GCS scores, Cognitive Reserve, MPAI) were the independent variables and patient initial visit fMRI maps were the dependent variables demonstrated significant correlation. There was a significant correlation between task-related activation and injury severity in regions of the bilateral middle temporal gyrus as well as in regions of the right frontal and right ventral temporal cortices (red). There was a significant correlation between task-related activation and Cognitive Reserve in a small region of the right ventral temporal lobe (blue). There was a significant correlation between task-related activation and general recovery in the right insular cortex and right parahippocampal gyrus (green). A p-value of .001 (uncorrected) and a cluster threshold of 50 voxels were employed. Age, gender and education were included as covariates.

Figure 3

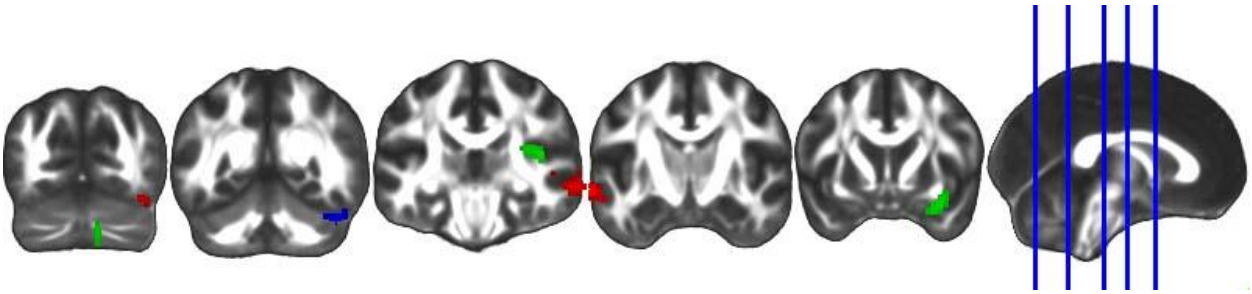


Figure 4: (a) This graph shows the percentage of TBI patients demonstrating different fMRI activation profiles at the initial scan. Patients are separated into two age groups (based on age at injury): patients ages 18-28 (n=8) and patients ages 29-52 (n=11). Normal= Patients exhibiting the normal pattern of activation seen in controls. (b) This graph shows the percentage of TBI patients demonstrating different fMRI activation profiles at the four year follow-up scan. Patients are separated into two age groups (based on age at injury): patients ages 18-28 (n=8) and patients ages 29-52 (n=11). At follow-up, all patients in the younger group exhibited a normalized activation pattern, while none of the patients in the older group exhibited normal activation profiles. Normal= Patients exhibiting the normal pattern of activation seen in controls.

Figure 4(a)

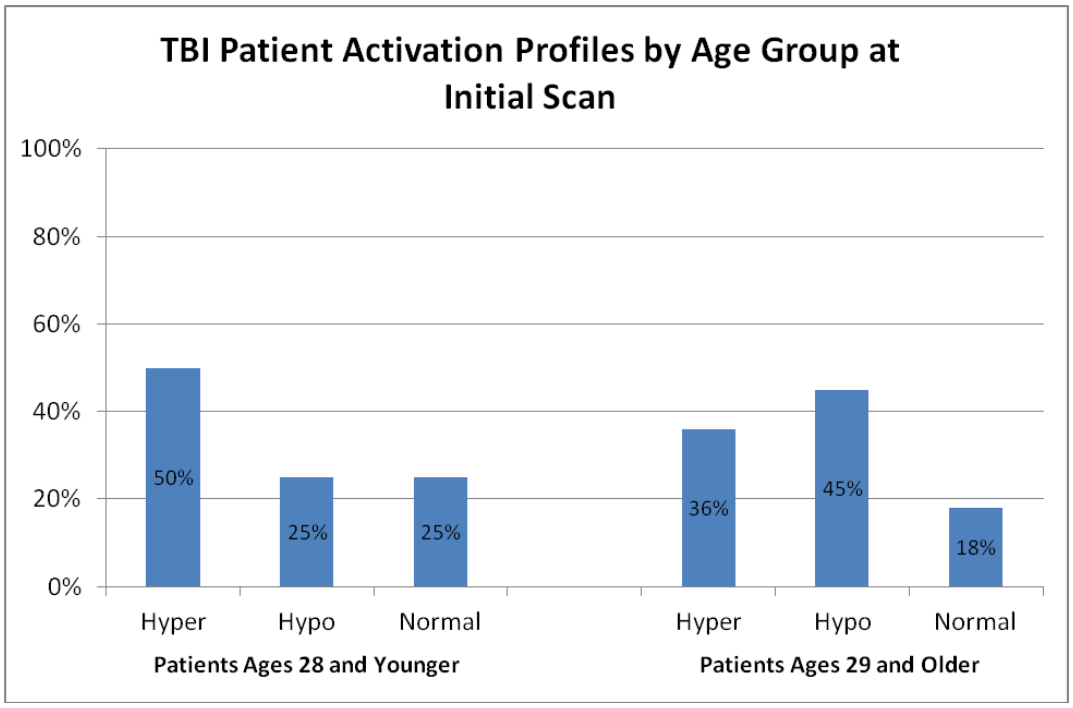


Figure 4(b)

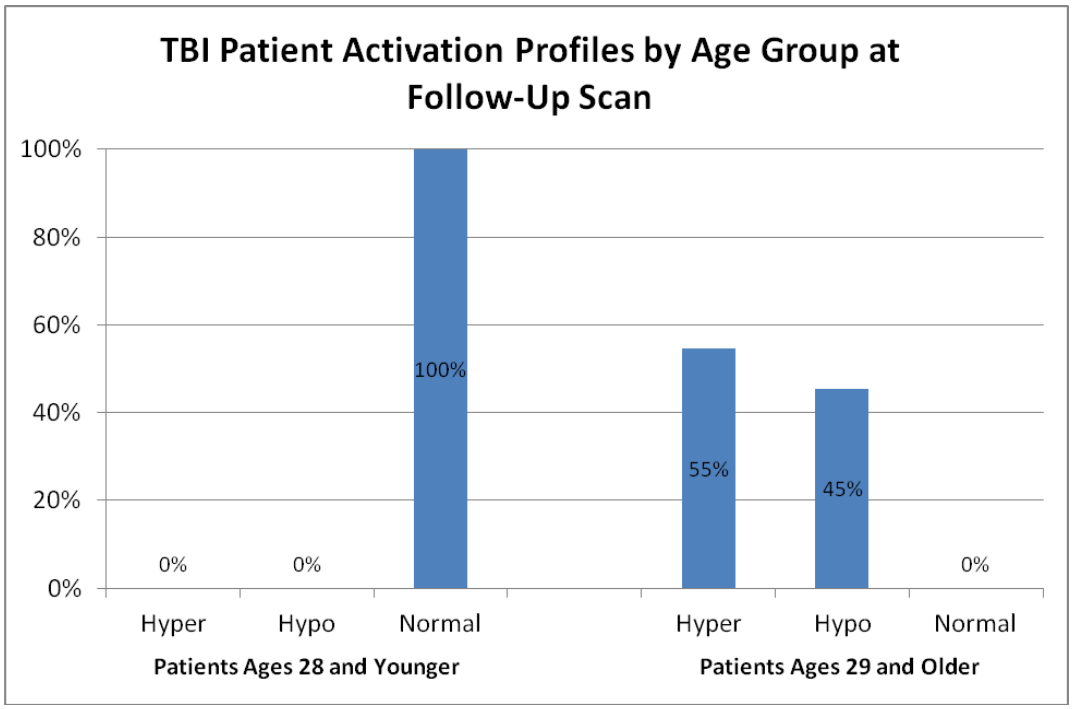


Figure 5: This figure shows the initial (left) and follow-up (right) fMRI scans of Patient 1. This patient had a GCS24 score of 7 and was initially classified as an hyper-activator (left). His follow-up scan shows a normalized pattern of task-related activation (right). Patient 1 was 23 years old at the time of his injury. All six patients under age 25 who demonstrated atypical activation at the initial scan demonstrated normalized activation patterns at follow-up. None of the nine patients over age 30 who demonstrated initially atypical activation exhibited normalized activation patterns at follow-up.

Figure 5

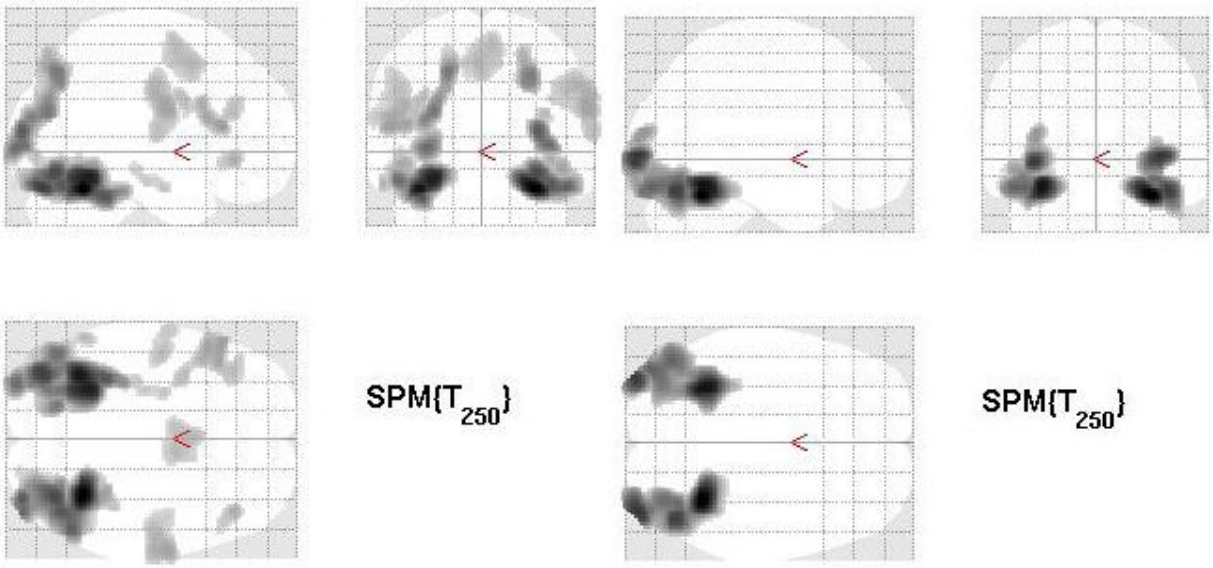


Figure 6: This figure shows the initial (left) and follow-up (right) fMRI scans of Patient 2. This patient had a GCS24 score of 10 and was initially classified as an hyper-activator (left). At follow-up, Patient 2 was still demonstrating an atypical activation pattern that included regions outside those normally active during the task. Patient 2 was 31 years old at the time of her injury.

Figure 6

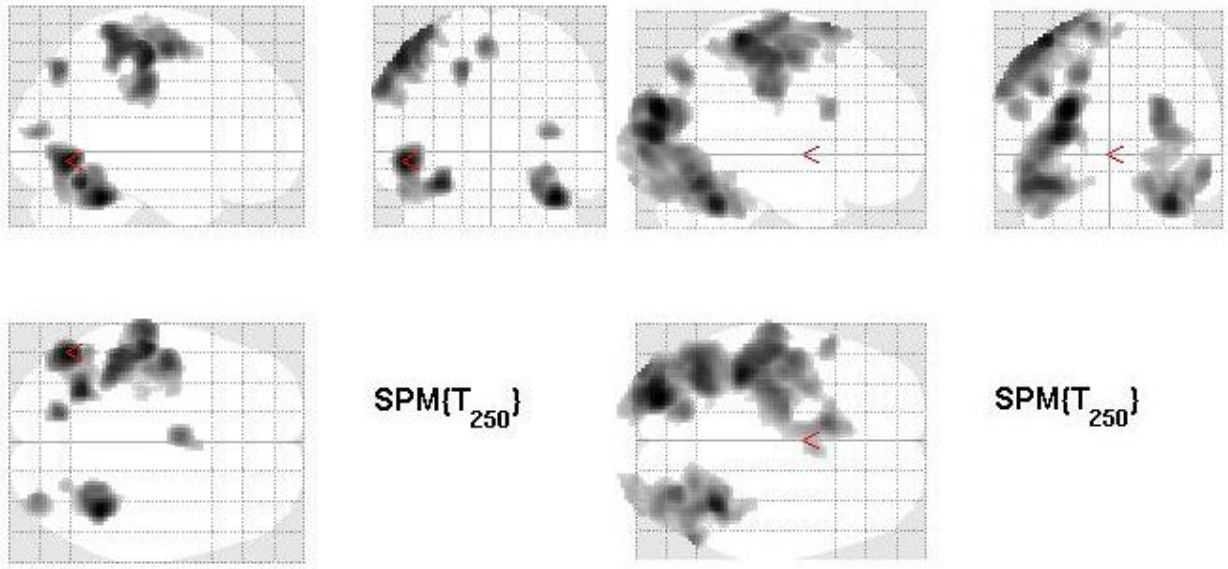


Figure 7: This figure shows the initial (left) and follow-up (right) fMRI scans of Patient 3, a 25 year old male. This patient had a low level of cognitive reserve and was initially classified as an hypo-activator (left). (Patient 3 Cognitive Reserve= -1.25; Mean TBI patient Cognitive Reserve= 0.00.) At follow-up, Patient 3's pattern of task-related activation had normalized.

Figure 7

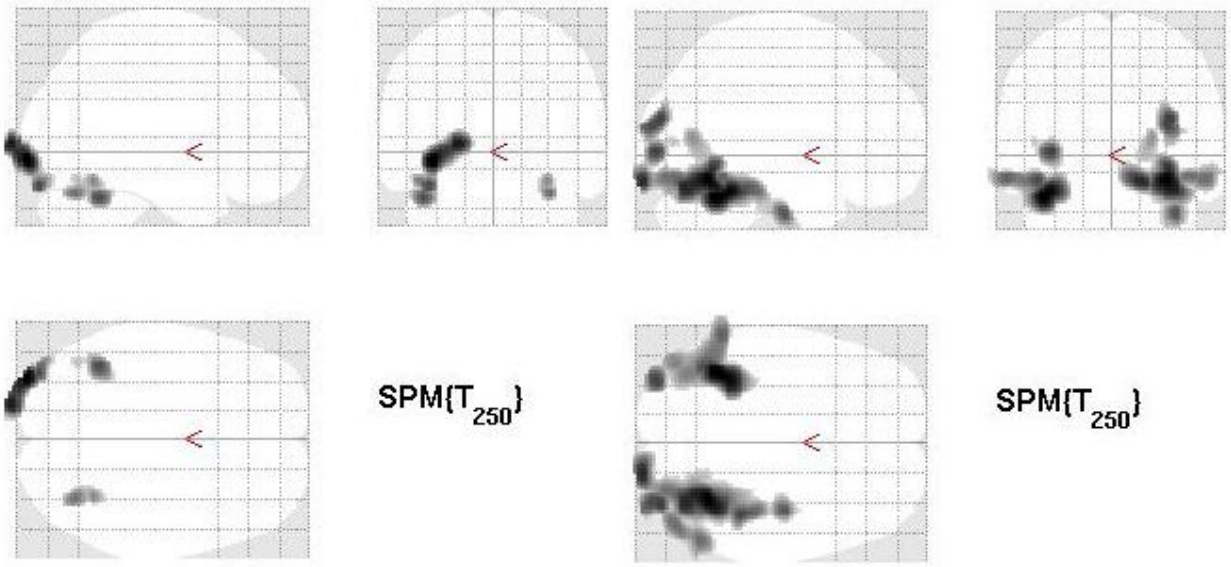
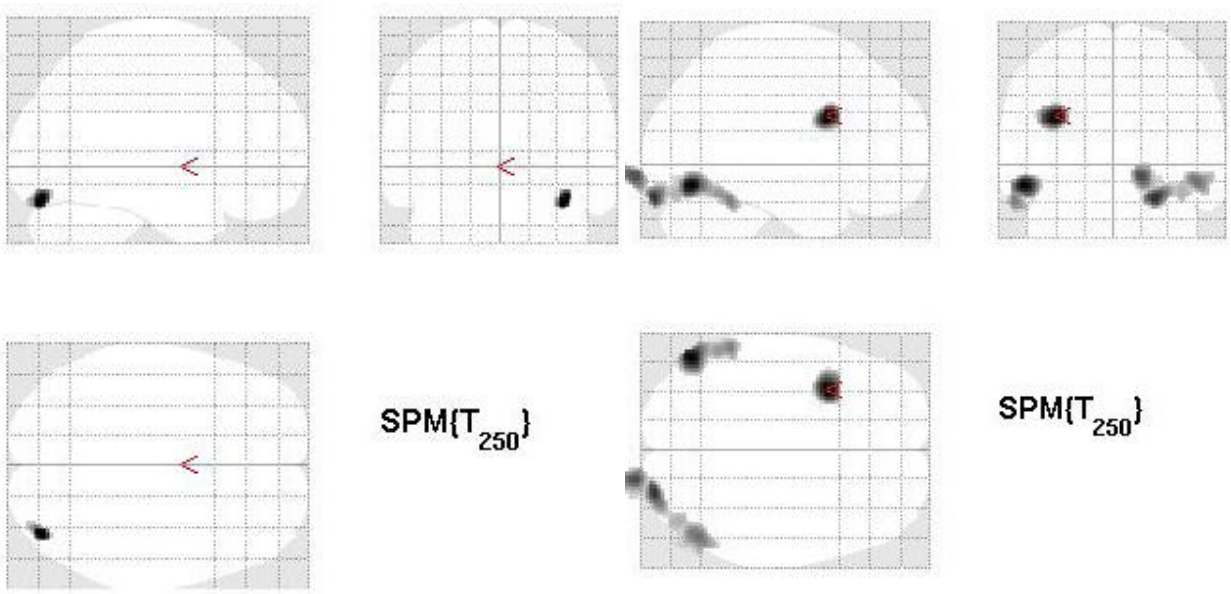


Figure 8: This figure shows the initial (left) and follow-up (right) fMRI scans of Patient 4. This patient had a low level of cognitive reserve and was initially classified as an hypo-activator (left). (Patient 3 Cognitive Reserve= -2.44; Mean TBI patient Cognitive Reserve= 0.00.) At follow-up, Patient 3's pattern of task-related activation remained atypical. Patient 4 was 49 years of age at the time of injury.

Figure 8



Chapter 5: Conclusion

In order to distill the findings in the three papers presented in the previous three chapters into a cohesive, meaningful contribution to neuroscientific knowledge of the TBI disease state, the final discussion includes the following: First, the three specific aims set forth in the introductory chapter of this dissertation and the findings addressing these specific aims are summarized. Second, the interrelation of these findings is explored. Next, the methodologies used in each of the previous three chapters are compared for their potential diagnostic and prognostic clinical value. Finally, recommendations for future research and a brief section of self-reflection are presented.

Summary of Findings

Summary of Results

The three specific aims set forth in the first chapter of this dissertation were: (1) Use diffusion tensor imaging to determine the duration and specific pattern of white matter change induced by TBI, and identify neuropsychological relevancies. (2) Use tensor based morphometry to determine the pattern of brain tissue degeneration following TBI and isolate correlations between brain volume loss and neuropsychological function. (3) Use functional magnetic resonance imaging to identify alterations in brain function following TBI and determine whether and how these alterations relate to patient characteristics and outcome.

Exploration of the first specific aim revealed that TBI patients demonstrate white matter damage throughout the brain that is persistent for several years post-injury. Furthermore, damage in the corpus callosum continues to progress for at least four years following TBI, while regions of the bilateral superior longitudinal fasiculus (SLF) demonstrate longitudinal improvements in white matter integrity. Secondary analyses indicate that myelin degeneration and repair are likely

driving these longitudinal changes. Longitudinal white matter recovery has never before been demonstrated in human TBI patients. Neuropsychological correlations demonstrate that poorer white matter integrity in the corpus callosum is associated with poorer performance on a finger tapping task, while higher white matter integrity in the SLF was associated with better performance on a visuomotor speed task. This study demonstrated that TBI patients experience concurrent white matter degeneration and repair in different brain regions, and that the integrity of white matter in these regions is relevant to simple motor performance.

The study addressing the second specific aim determined that TBI patients experience volume loss in diffuse regions throughout the brain during the first year post injury, and continued pervasive volume loss in brain white matter that continues for at least four years post-injury. No previous study has assessed regional volume loss in TBI patients beyond the first year post-injury, and late phase volume loss in white matter has never been previously observed in this population. Neuropsychological correlations indicate that cognitive tasks (visuomotor speed tasks and tests of cognitive control) can predict subsequent volume loss, and the regional volume loss and cognitive improvement sometimes correlate (visuomotor speed tasks). This demonstrates the prognostic value of neuropsychological tasks, which is well known and employed clinically, as well as the fact that volume loss likely co-occurs with ameliorative processes. The primary value of this study is that it demonstrates the occurrence of pervasive late-stage white matter volume loss, and indicates that this volume loss may not be entirely degenerative.

The final study seeking to tackle the third specific aim demonstrated that TBI patients demonstrate much greater variation in functional activation to a memory encoding task than healthy participants. While there was no clear group effect of TBI, two general patterns emerged:

First, TBI patients with low levels of cognitive reserve (an index of mental robustness based on age, educational attainment and baseline intelligence) tended to demonstrate significantly reduced task-related activation. Second, patients with the most severe injuries tended to exhibit much greater task-related activation compared to controls, and activation spread to regions not involved in the task among healthy individuals. These two qualitatively distinct patterns helped to explain why no group-wise effect was observed. The longitudinal component of this study showed that at the four-year follow-up, all patients under age 28 exhibited a return to normalized functional activation, while all patients ages 28 and over at the time of injury exhibited functional activation patterns that were still atypical four years post-injury.

Context and Interrelation of Findings

The research presented in this dissertation is part of a larger body of work that has been completed in our lab. Early work providing evidence of non-specific white matter degeneration and regional volume loss following injury inspired the decision to conduct a mutli-visit study and investigate whole-brain patterns of change in white matter integrity and volume loss longitudinally (Bigler et al., 1997; Blatter et al., 1997; Gale et al., 1995). These studies provided the basis on which the longitudinal studies presented in this dissertation were founded. Furthermore, prior work in our lab on the same cohort of individuals described in this demonstrated the need for the longer longitudinal studies presented herein. One previous paper (Trivedi et al., 2007) examined brain volume change during the first year post-injury, and demonstrated significant global volume loss. This work lead to the questions driving the TBM study, specifically whether volume loss continued beyond the first year post injury and what the precise pattern of this volume loss would be. Another previous paper (Bendlin et al., 2008) demonstrated significant differences in white matter integrity in large regions throughout the

brain closely following injury, and indicated that white matter integrity continued to decline during the first year post-TBI. The DTI study included in this dissertation was an extension of this work that included an additional time point to investigate how white matter change continued to develop beyond the first year post-injury.

In addition to connecting with earlier work, the three studies described above should be considered in relation to one another. The three studies described above indicate that TBI is both a traumatic event with immediate neurological repercussions and the instigation of a persistent disorder with both recuperative and degenerative elements. The first interrelation that should be appreciated is that volume loss observed in white matter and co-occurring improvements in white matter integrity are likely results of the same cellular processes. While it is not possible to ascertain the specific common mechanism underlying these changes, animal research indicates that autophagic processes are important to neurological recovery following TBI. The removal of cellular debris and dysfunctional axonal tracts would lead to both volume loss and enhanced white matter cohesion. Therefore, it is likely that autophagy contributes to the primary findings in each of these studies. It should also be noted that volume loss is likely not an exclusively degenerative process. Neuropsychological results from the volume loss study assessing the second specific aim indicate that regional volume loss correlates with functional improvements. This bolsters that case for recuperative autophagy, however no animal studies have examined cellular changes for several years post-injury so no definite conclusions can be drawn.

Secondly it should be noted that continued structural reorganization is necessary for the gradual functional improvements observed in some TBI patients. Patients demonstrating atypical activation at two months post-injury and then returning to normalized activation patterns at the four-year follow-up likely underwent a combination of recuperative processes that included

white matter healing, autophagic volume loss and functional reorganization. The changes in white matter integrity and volume loss observed in the other two studies presented are likely reflective at least partially of these processes.

Most generally and most importantly these studies all demonstrate that TBI-induced brain change does not reach a "chronic" phase at six months or one year post injury, but rather continues to develop for several years. These modalities, when used in concert with one another, provide greater space for postulating mechanisms than a single modality could alone. It is still not known when the TBI brain returns to a state of structural and functional equilibrium. Given the fact that a TBI early in life is associated with increased probability of developing dementia many decades later, it is possible that a chronic phase is never truly reached, but rather a slow degeneration is initiated. Regardless, further human research and animal studies will be necessary to develop more effective treatment trajectories and new therapeutic targets for TBI patients.

Comparative Prognostic Value of Neuroimaging Techniques

Utilizing three different neuroimaging modalities to longitudinally assess the same cohort of TBI patients facilitates methodlogical comparisons that may have clinical relevance. While the primary focus of this work was to make neuroscientific advances that improve understanding of the TBI disease state, secondary assessment of clinical potential, while not a planned part of this scientific work, may also be of use. Therefore, the following comments should be appreciated in a context of primary scientific advancement which seeks to inform secondary future clinical applications. Diffusion tensor imaging enables an immediate analysis of white matter damage which can provide information that is complimentary to a traditional structural scan. Furthermore, the pattern of white matter damage might give insight into the potential for recovery. For example, if the pattern of continuing corpus callosum degeneration observed in our study is upheld, damage to this structure might signify a particularly poor prognosis. For these reasons, it is likely that once the long-term neurological trajectory of TBI is better understood DTI will have strong diagnostic and prognostic value.

The nature of tensor-based morphometry necessitates longitudinal data. Therefore, TBM would not be a plausible diagnostic or prognostic tool immediately after injury. However, using TBM at a six month or one year evaluation could likely provide useful information. In our study, greater initial damage predicted greater subsequent volume loss, however regional volume loss and improved performance on tasks utilizing said regions also correlated. Therefore, in patient assessment correlations between regional volume loss and changes in cognitive and motor measures might be useful in determining the how adaptive volume loss exhibited by a particular patient is. Overall, however, the diagnostic and prognostic value of TBM for individual patients is relatively low. The value of this technique to the treatment of TBI patients will likely be

limited to the power of this tool to provide new information about that TBI disease state via research studies.

Functional MRI can be used shortly after injury, however our study indicates that a normal pattern of functional activation close to the time of injury does not predict a normal scan at follow up nearly as well as a patient's age. Very young patients present normally at follow-up while patients over the age of 30 do not, regardless of initial status. Initial fMRI scans also did not predict measures of global outcome or future memory function. While larger studies are necessary to confirm that our findings can be generalized, our study indicates that fMRI of a memory task is not a strong prognostic tool. However, fMRI could be effective in tracing recovery. Overall, the studies presented in this dissertation indicate that DTI has the most potential for use a diagnostic and prognostic tool for TBI patients. TBM and fMRI could both provide additional information in the tracing of recovery.

Contributions to Knowledge

Each of the three studies presented in this dissertation includes a primary unprecedented finding. In brief, these novel findings are:

(1) Traumatic brain injury patients experience regional white matter recovery that takes place over the course of several years following injury.

(2) Traumatic brain injury patients demonstrate pervasive volume loss in brain white matter for several years post-injury.

(3) Traumatic brain injury in persons ages 30 and over leads to persistent functional differences.

These results are unique and clinically relevant individually, but together they provide compelling evidence for the growing consensus among researchers that TBI should be viewed as the acquisition of a progressive neurological disorder with both degenerative and ameliorative facets. Understanding that the TBI disease state includes elements of long-term neurological recovery may help develop more effective treatments. In particular, the progressive nature of the TBI condition underscores the need for treatments that continue well beyond the six months to one year for which they are generally administered. Capitalizing on the long-term malleability of TBI brain could lead to significant improvements in the effectiveness of available TBI treatments.

From a societal perspective, TBI is a leading cause of disability that renders young people unable to work and live independently. While longer term treatment would represent an initial investment, if new treatment options were proven effective and patients were able to continue making gains for several years post-TBI, the percentage of TBI patients relying on

long-term personal care and public assistance would likely decrease. From an individual perspective enhanced treatment options could provide TBI patients with a significantly enhanced quality of life made possible by a more complete recovery. While much more work needs to be done to verify these findings and develop translational approaches to utilize this new knowledge in a clinical setting, the studies presented in this dissertation are part of a growing body of work that is enabling researchers and clinicians to gain a fuller understanding of the TBI disease state.

Limitations

A few limitations of these studies and their collective interpretation deserve mention. First, the sample size in these studies is small, and all studies were conducted on the same cohort of individuals. This means that if the group of individuals tested were in any relevant way atypical, these results may not generalize to the TBI population as a whole. Furthermore, it is not possible to determine the mechanisms underlying the changes observed through imaging data alone. While inferences can be made, in the absence of complimentary cellular-level studies in animals and biological data obtained from patients said inferences are of limited usefulness. Additionally, none of the studies in this dissertation contained data about the rehabilitation programs (or lack thereof) that patients underwent. This data could be critical in determining which patients demonstrate the recovery patterns observed in some of the included studies.

Recommended Future Directions

Future studies should seek to follow larger samples of TBI patients, possibly for longer periods of time. TBI patients are an itinerant population who are difficult to track long term, but larger sample sizes are necessary to determine whether observed patterns of degeneration and recuperation can be generalized. Following patients for a longer period of time could be useful in determining whether a "chronic" state of equilibrium is ever reached following TBI. Further work should also seek to collect complimentary biological data from patients. Using lumbar punctures to estimate neurotransmitter and other neurochemical levels could be of great use in bettering our understanding of the TBI disease state and recovery process. The long-term structural and functional changes observed in the studies presented in this dissertation are undoubtedly accompanied by chemical changes, and these changes have never been assessed in a long-term longitudinal fashion.

Other interesting questions could assess whether and how recovery from TBI relates to neurogenetics, synaptogenesis and stem cell proliferation. While there is no current method that could accurately track these processes in humans, future methods will likely be able to trace these processes. If new PET tracers are developed, for example, that enable researchers to visualize the creation of new synapses, Wallerian degeneration, or proliferation of stem cells in the human brain new insight regarding the mechanisms underlying TBI recovery may be identified. With relation to the studies described herein, new tracers could be used to determine whether continued white matter degradation (both in terms of integrity and volume loss) was a result of Wallerian degeneration, apoptosis, necrosis, autophagy or other processes and whether white matter healing was associated with the proliferation of new oligodendrocytes. Genetic

profiling could also be used to identify genes associated with enhanced outcome, which may in turn be useful in the identification of therapeutic targets.

Future research should also further investigate the effect of age (particularly in patients in their twenties compared to their thirties) on recovery. The fMRI study presented in this dissertation indicates that patients under the age of thirty eventually return to a normalized pattern of activation to a memory task, while patients over thirty do not. Most studies of aging have assessed how people under ages forty-five or fifty compare to persons in their sixties and beyond. This study indicates that a change in recovery capacity may occur much earlier than previously thought, and future studies of TBI should include assessments of how these two age groups fair in recovery. This could be particularly pertinent to timely issues such as traumatic brain injury among veterans and athletes, many of whom cross the thirty year mark during their time in the service or playing professional sports. It is true that chronic small TBIs and single more severe TBIs have different characteristics but the possibility of mechanistic overlap is strong and should be further investigated.

Also, studies should try to differentiate the contributions of cognitive reserve (as conferred by educational attainment and intelligence) compared to brain reserve (associated with youth, physical fitness and generally enhanced neurological health) to enhanced prognosis. Cognitive reserve and brain reserve both confer resilience to initial damage and are associated with enhanced long-term outcome, however the mechanisms underlying this association are poorly understood and the two categories of resilience may lead to divergent recovery paths. Specifically, it is possible that those with higher levels of brain reserve will be able to effectively clear away damaged tissues in the injured brain while healing salvageable neurons and generating new synapses allowing the patient to slowly return to using his or her brain in a way

similar to how it was used pre-injury. Improving general health through sleep, nutrition and exercise may be the best way to maximize recovery for this type of patient. Those with lower levels of brain reserve and greater cognitive reserve, however, may have to adapt by utilizing different cognitive strategies and different brain regions to complete the same tasks. Cognitive training may be particularly useful to these patients. Both cognitive and brain reserve contribute to resilience to TBI-induced damage and both are associated with enhanced outcome. However, future work aimed to further develop our understanding of how these concepts relate to TBI could facilitate the development of more tailored treatments.

In addition to larger and more in-depth observational studies, comprehensive intervention studies could be of great use. For example, a study in which patients received a combination of tailored pharmacotherapy, behavioral and cognitive rehabilitation, and psychotherapy for five years post injury compared to those who received only six months to one year of such a combination therapy could help to determine whether longer term treatment resulted in better long-term results. Another possibility would be to investigate the influence of lifestyle factors (physical fitness and exercise, nutrition, alcohol consumption, sleep) on long-term outcome. Clinicians may be able to capitalize on the continued malleability of the TBI brain to facilitate better general long-term recovery, and intervention studies are necessary to determine the effectiveness of protracted treatment. Finally, animal studies examining long-term cellular-level changes that occur following TBI could be important for developing new targets for pharmaceutical treatment of TBI. Specifically, such studies could be useful in identifying mechanisms of plasticity and synaptogenesis and investigating late-phase autophagy and synaptic pruning. The work presented in this dissertation was meant to address a gap in the understanding of long-term outcome following TBI at the global level, however the new

knowledge presented herein creates a new gap. It is unknown what cellular and molecular level changes underlie continuing large-scale changes described here, and new work with PET, neurochemical assays, and animal models of injury will all be necessary to understand the mechanisms of the global changes induced by TBI.

Self-Reflection

As is customary in doctoral dissertations, I would like to conclude with a brief exploration of what I have learned, both about science and about myself. The most critical thing I have come to appreciate about the world of research science in general is that a scientific finding regardless of how eloquently unearthed should not be looked upon as unalterable fact. Two or more valid studies may provide contradictory results for any number of reasons. Instead, it is necessary to look at the body of evidence concerning a particular topic and consider it in a larger context. Science describes the natural world with an ever increasing degree of accuracy, but the current understanding of any cutting edge topic is always a work in progress.

In terms of my own growth during my time as a graduate student, I've gained many things: a deeper understanding of the human brain and mind, the ability to self-motivate and follow several year-long projects to completion, and the capacity to synthesize a large body of facts into a meaningful whole. As I approach the culmination of this invaluable experience I feel empowered with a strong sense of my ability to accomplish any goal, from the identification of a problem through all the planning, research, communications, exertion and logistics necessary to craft and enact a solution. For me, this sense of empowerment has been the greatest reward of the doctoral process.

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