STRUCTURAL BRAIN DIFFERENCES IN EX-ATHLETES WITH MULTIPLE CONCUSSIONS

by

DOUGLAS PRYSE TERRY

(Under the Direction of L. Stephen Miller)

ABSTRACT

Recent research has suggested that mild traumatic brain injuries, colloquially referred to as concussions, may alter brain structures and influence cognitive functioning later in life. The purpose of this study was to compare neuroanatomical structures between former high school football players with a history of multiple concussions and former high school football players without a concussive history using traditional magnetic resonance imaging (MRI) and diffusion tensor imaging (DTI). Further, this study examined if concussion status and brain-based metrics were associated with cognitive functioning. It was hypothesized that those with concussive histories would have smaller overall brain volumes, hippocampal volume, and anterior cingulate on MRI scans, as well as reduced white matter integrity on DTI scans compared to the non-concussed controls. Participants were right-handed males currently ages 40-65 recruited from the community. Results failed to show volumetric differences between the two groups on MRI scans, though overall gray matter volume was associated with global cognition in the overall sample. However, DTI scans showed reduced fractional anisotropy across multiple brain regions, suggesting diffuse white matter differences. Tractography analyses of *a priori* regions of interest showed increased radial diffusivity in the corpus callosum of the concussed group as compared

to the control group. This is suggestive of myelin-related changes, such as a disruption in initial white matter formation during development given that the concussions sustained were during adolescence. There results suggest that there are subtle neuroanatomical differences in the brain of people who have sustained concussions early in life, but that these differences have not manifested in a functional deficit.

INDEX WORDS: concussion, mild traumatic brain injury, structural imaging, MRI, DTI

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Overview of study

Over the past decade, neuroscientists have examined mild traumatic brain injuries, more commonly referred to as concussions, using *in vivo* imaging modalities to quantify their pathophysiological effects of and relate said effects to long-term outcomes. The majority of these studies have sampled participants from either professional/collegiate sports teams, emergency departments/medical records reviews, or veterans who experienced head trauma in the line of duty. However, sports concussions at the non-professional level are some of the most frequent types of concussions, as teenagers and young adults are at especially high risk of experiencing a concussion during athletics (CDCP, 2010). Unfortunately, little research has examined the consequences of these sports concussions and how they affect brain integrity later in life.

In this study, we analyzed two types of neuroimaging scans in a sample of former athletes currently ages 40-65 who experienced at least two concussions while playing high school football. Using 3D FSPGR MRI scans, we explored volumetric differences in the whole brain as well as in *a priori* regions of interest, including overall brain size, overall white matter, and hippocampi by comparing the participants who experienced mild traumatic brain injuries to their non-concussed former teammates. Using diffusion tensor imaging, we quantified the integrity of white matter tracts in the brain and compared them to the control group to assess potential white matter pathology related to the progression of time in the concussed brain. We also explored the associations between cognitive functioning and these brain indices.

Breadth of the issue

Approximately 1.7 million people experience a Traumatic Brain Injury (TBI) in the United States each year, with over 75% of these classified as a mild traumatic brain injury (mTBI; CDCP, 2010; Faul 2010). This number may be an underestimate of the actual number of mTBIs experienced as it does not take those who sought medical treatment in a primary care setting or those who did not seek medical treatment into account (Langlois, Rutland-Brown, & Wald, 2006). The economic impact of mTBI is substantial, accounting for approximately 44% of the $56 billion annual cost of TBI in the United States (Thurman, 2001).

A simplified view of a mTBI, or concussion, is that it is a brain injury with acute physical symptoms, such as headaches, dizziness, fatigue, irritability, anxiety, and impaired neuropsychological functions such as reduced attention, concentration, and memory problems (McCrory et al., 2009; Arciniegas et al., 2000; Hall, Hall, & Chapman, 2005). For most people, these symptoms spontaneously resolve without systematic treatment within hours, days, or weeks (McCrory et al., 2009; Guskiewicz et al., 2003; Schretlen & Shapiro, 2003; Frencham, Fox, & Mayberry, 2005), but 8-30% of people may be affected by persistent post-concussive symptoms that do not resolve three months post-injury (e.g., Alexander 1995; Bazarian et al. 1999; Bigler 2008). These long-term physical, psychological, and physiological difficulties are commonly known as Post-Concussive Syndrome (PCS). Explaining the etiology of these symptoms in the absence of radiological abnormalities has led some to consider psychogenic origins, as having a premorbid psychological diagnosis was shown to be one of the most predictive determinants of developing PCS after a concussion (Al Sayegh, Sandford, & Carson 2010). However, it is possible that these symptoms are the result of pathophysiological or

neurological processes, but the current imaging techniques are not sensitive enough to reveal brain pathology (Huisman et al., 2004; Niogi and Mukherjee, 2010).

Lack of neurological and radiological evidence of PCS in mTBI is not the only problem. Medically diagnosing an mTBI immediately post-injury can also be difficult, as the brain is frequently deemed to be normal on traditional brain imaging modalities such as computerized tomography (CT) and magnetic resonance imaging (MRI) (e.g., Bazarian et al. 2007; Hughes et al. 2004, Iverson et al., 2000). Thus, the diagnosis of an mTBI is typically based on self-reported clinical and cognitive symptoms following head trauma. This can be problematic as the symptoms of an mTBI are nonspecific and can occur in a variety of other medical and psychological conditions (Stein and McAllister, 2009).

However, there may be ways to improve neuroimaging methods to more sensitively detect brain abnormalities sustained related to an mTBI. There is evidence that there are, in fact, pathophysiological changes associated with an mTBI, as autopsy studies have suggested that some who have experienced an mTBI with negative radiological findings have microscopic axonal injuries (Bigler, 2004; Blumbergs et al., 1994). These changes are likely the result of the mechanical impact of the head injury. Rapid acceleration and deceleration forces related to the concussive event are thought to stretch, twist, and shear axons or cause alterations in cell function that manifest as the acute symptoms of an mTBI (Blennow, Hardy, and Zutterberg, 2012).

Neuropathology of mTBI

There are two types of brain damage due to trauma: focal damage and diffuse injury. Focal damage includes contusions, lacerations, and intracranial bleeds (e.g. hematoma and hemorrhages; Blennow, Hardy, and Zutterberg, 2012). These types of injuries are common in

moderate and severe TBI. Diffuse injury is more characteristic of mTBI as it can occur without skull fracture or direct impact with the brain. Diffuse axonal injury (DAI) is the main mechanism for an injury of this type, which involves the shearing of axons due to the acceleration/deceleration and rotational forces of the impact. The stretching of the axonal cell membranes cause a "deregulated" flux of ions, including an efflux of potassium and an influx of calcium (Blennow, Hardy, and Zutterberg, 2012). This triggers an enhanced release of excitatory neurotransmitters, namely glutamate, which bind to receptors and cause further calcium influx and widespread suppression of neurons with glucose hypometabolism (Giza and Hovda, 2001). To restore the ionic balance, membrane pumps increase their activity, but this further depletes glucose, causes calcium influx into mitochondria, and impairs oxidative metabolism. These processes may cause acidosis and edema (i.e. swelling; Barkhoudarian, Hovda, and Giza, 2011; Giza and Hovda, 2001).

These cellular level changes may cause DAI, which has been detected within hours of the trauma. Such disruptions in axon connections and axonal swelling may eventually cause Wallerian degeneration due to the breakdown of microtubules triggered by excessive cellular calcium (Giza and Hovda, 2001). Neuroimaging studies have shown that the extent of DAI after an mTBI is related to increases in cognitive deficits post-injury (Lipton et al., 2008). Studies of those with repetitive head injuries have reported increased rates of neurofibrillary tangles, tau pathology, amyloid precursor protein, and amyloid-beta (Blennow, Hardy, and Zutterberg, 2012). The buildup of these proteins in the brain are traditionally associated with Alzheimer's disease, but are shown to be present in a variety of different neurodegenerative disorders (McKee et al., 2009). The mechanism about how the formation of these proteins is related to chronic

concussions is not yet fully elucidated, but likely involves a series of complex biological cascades triggered by neurofilament and microtubule deficits.

Magnetic Resonance Imaging (MRI)

MRI became popular in the 1980s as an alternative to CT imaging as it allows for better spatial resolution and does not involve the potentially harmful radiation associated with x-ray technology. The basic principle behind MRIs is that a static magnetic field aligns the hydrogen nuclei (i.e. protons) in the direction of the field. Then, a radiofrequency (RF) pulse excites the protons. By properly modulating the basic field and the timing of the pulses, the relaxation of the protons to their non-excited state will emit a signal that is spatially encoded and results in images. The brightness (e.g. white, gray, black) of the image is directly associated with the amount of time it takes the proton to relax from its excited to its non-excited state, which differs by tissue density.

As this technology has advanced, MRI has become a more and more powerful technique. Magnet strengths have increased and RF equipment has become more sophisticated, which allow for higher resolution images to be collected over shorter amounts of time. It also allows for researchers and clinicians to differentiate between gray and white matter on a brain scan, which is typically not possible on a CT image. MRI scans are more sensitive in detecting white matter shearing, small subacute hemorrhages, small contusions, and small foci of axonal injury as compared to CT (see Niogi and Mukherjee, 2010). MRI can also better differentiate brain and CSF as well as edema as compared to CT scans (Johnston, et al., 2001), which are important considerations when evaluating mTBI using imaging modalities. In a study examining neuroimaging findings after a clinical diagnosis of mTBI that had negative CT scan findings,

abnormalities consistent with diffuse axonal injuries were found in 30% of MRI scans (Mittl et al., 1994).

MRI studies in mTBI

The majority of the MRI studies examining TBI are related to its higher sensitivity in detecting pathophysiological changes compared to CT scans. More recently, investigators have used MRI technology to examine more subtle, non-diagnostic volumetric and morphological differences in brains of people with mTBI as compared to non-injured counterparts. These studies have been carried out with various methodologies, examining the brains of people recently affected by a brain injury to those many years after the injury occurred. Many studies included those with mTBIs in studies with more severe TBIs and did not differentiate between the groups. Notable findings will be presented in the following section.

In a clinical context, MRI has been associated with better detection of brain abnormalities after mTBI compared to CT scans. In patients with a single mTBI complicated with loss of consciousness and post-traumatic amnesia, neuroradiologists detected intraparenchymal lesions in 75% of the cases (Lee et al., 2008). Not only is the percentage of abnormalities greater for MR images than that found in CT scans, but this helps to show that there are likely acute brain abnormalities that are detectable using MRI. Hofman et al. (2001) reported a similar percentage of patients had lesions on MRI scans acutely after an mTBI in an emergency department setting. They also showed that when scanned again at six months post-injury, patients with abnormal MRIs had a greater rate of brain atrophy than those without MRI abnormalities (Hofman et al., 2001). In collegiate hockey players, global volume reductions were noted after a concussion at 2-weeks and 2-months (Jarrett et al., 2016). However, in a recent study examining ventricular volume, gray matter volume, and cortical thickness during the acute (i.e. within three weeks) and

semi-acute phase of recovery (i.e. four months later), there were no significant differences between the mTBI group and a matched control group (Ling et al., 2013).

Another imaging modality is called Texture Analysis, which quantifies irregular cortical surfaces indicative of microstructural brain injury not perceptible by visual inspection (e.g. smoothness vs. roughness). People diagnosed with an mTBI in a medical setting were scanned within three weeks of their injury. Results showed within group texture differences between the left and right mesencephalon and between the hemispheres in WM, especially in the level of corona radiata and between different segments of corpus callosum (Holli et al., 2010). Further, the controls were more symmetric in texture than the patient group, suggesting that subtle brain changes may be associated with mTBI and detectable during the acute recovery period.

When examining chronic changes in brain volume (i.e. greater than six month post-injury), many studies incorporated samples that were heterogeneous with respect to TBI severity. However, several investigators found significant volumetric differences in various brain regions compared to control participants. At an average of 21 months post-injury, Gale et al. (1995) showed that those with a TBI had decreased whole brain volume compared to control participants. Further, they showed that patients had reduced corpus callosum volume, increased ventricle volume, and a decreased fornix-to-brain ratio that was significantly correlated with multiple neuropsychological measures. However, the patient group in this study was only 41% mTBI, with the remainder of the participants having experienced a more severe brain injury.

A longitudinal study that combined mTBI (79% of patient group) with moderate TBI (21% of patient group) showed that those with a TBI had a greater rate of whole-brain atrophy during the 11-month testing interval as compared to control participants (MacKenzie et al., 2002). Further, the rate of atrophy was greater for the patients that experienced a loss of

consciousness with their injury. This conclusion was upheld in a study examining symptomatic patients with an mTBI at an average of two years post-injury, which showed that the mTBI patients had a higher rate of atrophy in forebrain parenchyma, cerebral white matter, and cerebellar volume as compared to controls at two different time points (Ross et al., 2012). A group of people with a documented mTBI in an emergency department setting exhibited global brain atrophy compared to a control group on longitudinal scans taken acutely after the mTBI and again at 1-year post-injury (Zhou et al., 2013). Region of interest analyses indicated reduced gray matter volume in the precuneus and white matter volume in the anterior cingulate, which correlated with memory and attention performance (Zhou et al., 2013). Cohen et al. (2007) used a cross-sectional method to examine people with an mTBI at various times post-injury (range: less than 9 days to 31.5 years) and well-matched controls. After adjusting for age, they showed that the mTBI group had a significant negative relationship between the time since injury and whole brain/gray matter volume on MRI as well as greater evidence of neuronal/axonal injury using MR spectroscopy (Cohen et al., 2007).

Novel methods to estimate brain atrophy have also been used. Ross et al. (2014) examined 26 patients with either a mild (n=24) or moderate (n=2) TBI with continued post-concussive symptoms at an average of 1.7 years post injury (time 1) and again several months later (time 2; average follow-up=0.7 years after time 1). They used these longitudinal MRI scans to estimate the size of the brain just prior to injury (time 0). Compared to a control group, the patient group had abnormally rapid atrophy in whole brain parenchyma and cerebral white matter between time 0 and time 1, while the cerebellum and brainstem enlarged rapidly over the first few months post-injury (time 0-time 1) and then diminished (time 1-time 2; Ross et al., 2014), suggesting that different brain regions are affected by a TBI in different ways. Tate et al.

(2014) examined cortical thickness on a sample of veterans who experienced a blast-related mTBI within 18-months of injury and found that the mTBI group exhibited cortical thinning in the left superior temporal and left superior frontal gyri, which were associated with abnormal language and audition scores on behavioral measures.

Looking at specific regions within the brain has also showed differences associated with mTBI. Monti et al. (2013) found that those who experienced a medically diagnosed mTBI before the age of 25 years old had smaller bilateral hippocampi compared to well-matched control participants at an average of 30 years post-injury. They also found that this sample did worse on a face-scene relational memory task and had less functional MRI activity in memory-related regions in the parietal and prefrontal cortices. However, these differences were not evident in younger samples after an average of four years post-injury (Monti, et al., 2013), suggesting that these subcortical changes may take place over a longer period of time.

Many of the studies presented thus far have been conducted in medical populations, where a concussion may occur at one discrete time point. However, athletes that play contact sports may have different brain changes in response to a concussion due to the accumulation of chronic impact to the head. In a sample of current college football players, participants that experienced a clinician-diagnosed mTBI had smaller bilateral hippocampi compared to a sample of non-concussed teammates as well as a sample of non-concussed non-athletes at a post-injury period ranging from 1 day to 4.5 years (Singh, et al., 2014). Additionally, the non-concussed football players had smaller bilateral hippocampi compared to the control group, suggesting that the sub-concussive impact associated with football may have an impact on brain structure. However, the authors also suggested that these hippocampal changes might be due to the increased stress associated with playing a collegiate sport. Higher stress levels would trigger the

release of glucocorticoids, which have also been associated with smaller hippocampal volumes (McEwin, 2001). The time players reported engaging in football was negatively correlated with hippocampal volume suggestive of a dose-response relationship (Singh et al., 2014).

More chronic changes have also been documented in sports-related mTBI. Cortical thinning of the right temporal lobe and left insula was seen in a sample of young adult athletes with multiple concussions who had not had a concussion in the past 6-months as compared to non-concussed controls (List, Ott, Burkowski, Lindenberg, & Floel, 2015). In a sample of former college athletes ages 50-75 who experienced between one and five concussions (average = 2.08) before their mid-20s, authors found cortical thinning in frontal, temporal, and parietal areas when comparing the previously concussed athletes to their non-concussed teammates (Tremblay et al., 2013). Authors also found abnormal enlargement of the lateral ventricles and worse memory performance for the injured group, which resemble patterns of abnormal aging reflected in neurodegenerative conditions often seen later in life.

The mTBI results resemble the findings from studies examining more serious forms of TBI, as research supports the idea that brain injuries occur on a continuum based on severity (see Bigler, 2013). Studies examining parenchymal volume loss show that brain atrophy is linearly related to severity of injury from mild to severe (Ghosh et al., 2009, Levine et al., 2008). This is likely due to the underlying physiological disruption also taking place on a spectrum given that metabolic pathways and inflammatory reactions are also shown to be directly related to injury severity (Rostami, et al., 2012).

Diffusion tensor imaging (DTI)

DTI is a form of diffusion weighted imaging (DWI), which is based on the motion of water molecules in brain tissue. This motion is heavily influenced by the speed of water

displacement due to the physical properties of different tissue types (i.e. gray matter, white matter, cerebrospinal fluid) as different tissues displace water at different rates. This method was initially used to assess acute ischemic events as it was thought that decreased diffusion was indicative of cytotoxic edema. Since then, it has been applied to TBI to quantify edema and reductions in white matter fiber bundle integrity (Assef and Pasternak, 2008; Niogi and Mukherjee, 2010).

DTI is an advanced technique in neuroimaging as it is able to assess white matter changes at the microstructural level in an *in vivo* way. This is an advancement from traditional CT and MRI technologies, which are limited to revealing only macrostructural changes in the brain. Axonal injuries thought to be associated with mTBI are likely better assessed at the microstructural level (Pierpaoli and Basser, 1996). Pathology is quantified in DTI based on the restriction of water flow based on the tissue being measured. For example, cerebral spinal fluid (CSF) in a ventricle is unrestricted in space, so water moves freely in every direction (i.e. *isotropic diffusion*). However, CSF near white matter fibers is restricted to the direction of the axon due to the organization of axon membranes' myelin sheaths, microtubules, etc. (i.e. *anisotropic diffusion*; Beaulieu, 2002)

To image and quantify the restriction of water movement, DTI acquires diffusion-weighted images along various orientations in order to calculate a "tensor" that describes the shape of the water diffusion along three principal axes (Melhem et al., 2002). Each tensor has three vectors (v1, v2, v3), which represent the three primary, orthogonal directions of water flow. Through various combinations of three eigenvalues ($\lambda 1$, $\lambda 2$, and $\lambda 3$), which are derived from the vectors and describe the strength of diffusion along the corresponding axes, several scalar indices

of diffusion can be calculated. These indices are thought to describe different properties of the surrounding white matter.

The different ways of charactering the diffusion tensor correspond to different microstructural abnormalities. Mean diffusivity (MD) is the average of all three eigenvalues and provides an overall measure of the degree of diffusion within the region. Increased MD is thought to indicate decreased WM integrity. This index corresponds to the *size* of the tensor. Fractional anisotropy (FA) indicates how directional, or anisotropic, diffusion is along the primary eigenvector. FA is a scalar value from 0 (i.e. isotropic) to 1 (i.e. anisotropic), and represents the ratio of the tensor's anisotropy to the whole tensor (Melhem et al., 2002). It is used as an index of overall white matter health (Beaulieu et al., 1996), with lower FA generally thought to reflect loss of white matter integrity that may reflect damage to myelin or axon membrane damage, or reduced axonal packing density, and/or reduced axonal coherence. However, MD and FA can be greatly affected in regions where there are crossing fibers (Alexander et al., 2007; Vos et al., 2012), thereby providing an inaccurate description of local diffusion characteristics.

Two further indices are thought to me more related to specific microstructural abnormalities. Axial diffusivity (AD) describes the principal eigenvalue ($\lambda 1$) and is thought to be related to axonal or changes in extra-axonal/extracellular space (Glenn et al., 2003; Beaulieu and Allen, 1994). In contrast, radial diffusivity (RD) is the average of the two other eigenvalues ($[\lambda 2+\lambda 3]/2$) and is thought to be associated with changes in myelination or glial cell morphology (Song et al., 2002, 2003, 2005).

DTI studies in mTBI

Given that DTI is sensitive to axonal injuries characteristic in TBI, it has been used to identify subtle brain abnormalities in mTBI acutely after an injury. An early study examined the FA and MD of several *a priori* brain regions thought to be associated with DAI within 24 hours of the injury in an emergency department setting (Arfanakis et al., 2002) and found reduced FA in parts of the corpus callosum and internal capsule compared to control participants. At a 1-month follow-up, the patients showed some "improvement" in FA values but not in all areas, suggesting the chronicity of DAI. At an average of four days post injury, another group of investigators found FA reductions in the corpus callosum, internal capsule, and centrum semiovale compared to controls, as well as higher MD values in the splenium of the corpus callosum (Inglese et al., 2005). However, in a chronic mTBI sample, Inglese et al. (2005) showed MD was decreased in the anterior limb of the internal capsule, again highlighting the importance of examining brain abnormalities over the course of the injury and its recovery. Miles et al. (2008) found similar findings at an average of four days post-injury. Further, they showed that FA reductions at baseline scanning predicted worse executive functioning six months after the injury. Lipton et al. (2009) showed lower FA and higher MD in frontal areas in patients scanned within two weeks post injury, which were also associated with worse executive functioning. Kumar et al. (2009) showed reduced FA as well as increased radial diffusivity in the corpus callosum in people with mild and moderate TBIs within two weeks of their injury. Similar differences have been found at the semi-acute phase of recovery. Cubon & Putukian (2011) showed increased MD in the left inferior and superior longitudinal fasciculi, fronto-occipital fasciculus, internal capsule, and thalamic radiations at one month post-injury.

Longitudinal analysis of collegiate athletes who sustained a concussion indicated that higher RD and lower FA were evident at both two days and two months post-injury in right hemisphere areas including the superior/inferior longitudinal fasciculi and internal capsule (Dettwiler, et al., 2014). In a sample of people with mTBIs who presented to the emergency department and were scanned at an average of 11 days post injury, there was reduced FA in patients with any intracranial lesion on traditional MRI/CT scans compared to controls, but no differences in DTI measures on patients with negative clinical scans (Yuh, et al., 2014). Further, poor functional outcome was associated with positive MRI findings.

However, it is important to note that some studies have failed to show differences between mTBI and control groups. For example, Zhang et al., (2010) did not find DTI differences in the corpus callosum using both whole-brain and ROI analyses on student-athletes with a concussion at 30 days post-injury compared to non-concussed controls. However, the mTBI group did show higher variability of FA in the genu of the corpus callosum. Lange et al. (2011) similarly found no FA and MD differences in people 6-8 weeks post-mTBI who sought emergency department treatment compared to control participants who sustained an orthopedic injury. However, they found that the mTBI group had higher MD in the splenium of the corpus callosum at a trend level. For a comprehensive review of DTI studies at the acute and semi-acute level, please see Dodd et al. (2014).

To make matters more complex, some studies have reported rather counterintuitive findings when examining DTI parameters. For example, Bazarian and colleagues (2007) scanned those with a concussion within three days of the injury and found decreased trace (a derivative of MD) in the internal capsule and increased FA in the corpus callosum. Authors suggested that these findings likely reflect axonal swelling that occurs early in the course of the axonal injury.

Additionally, lower trace values were correlated with more post-concussive symptoms and worse cognitive functioning at the time of scanning and at one month post-injury (Bazarian et al., 2007). To further explain these results, Kou et al. (2010) posited that increased FA and decreased MD indicate cytotoxic edema due to axonal swelling and more restricted water flow, which may lead to a poor clinical outcome. Alternatively, lower FA and higher MD may indicate vasogenic edema, which will likely resolve over time. Reduced FA and increased MD have been found in other studies at the acute phase of injury recovery in the corpus callosum and corticospinal tracts (Henry et al., 2011; Mayer et al., 2010).

In an effort to clarify these discrepant findings, Lipton et al. (2012) examined intra- and inter-individual patterns of FA in people who experienced an mTBI within the past two weeks. Patients and control subjects were scanned three times: within two weeks of injury, 3 months post-injury, and 6-months post injury. They found that most participants both had areas of abnormally low and abnormally high FA. Almost all patients (i.e. 32/34 participants) had lower FA in at least one brain region within two weeks of the injury as defined by $p<0.05$ and more than 100 significant voxels that formed a contiguous cluster. However, the same ratio of patients (32/34) also showed increased FA in at least one brain region within two weeks of injury (Lipton et al., 2012). Longitudinal analyses indicated that the number of abnormally high FA voxels peaked at the three-month follow-up and declined by 6 months, whereas the number of abnormally low FA voxels decreased as the time since the injury increased. Authors concluded that these inter-individual differences may be accounted for by individual differences in anatomy, vulnerability to injury, and mechanism of injury. Further, they suggested that a higher FA may be related to a compensatory mechanism or plasticity in response to injury rather than a

direct manifestation of brain injury (Lipton et al., 2012). Croall et al. (2014) also found this pattern when examining mTBI acutely and at one year post-injury.

Several investigators have also studied the chronic changes associated with white matter in mTBI. At an average of nine years post-injury, Kraus et al. (2007) reported that those with an mTBI showed lower FA and higher AD values in the corticospinal tract, superior longitudinal fasciculus, and sagittal striatum compared to control participants. There was no evidence of RD changes in mTBI, suggesting that the damage of chronic mTBI may be related to axonal integrity but not myelin processes. A whole-brain analysis of white matter integrity in patients who experienced an mTBI eight months to three years earlier showed lower FA and increased MD in the corpus callosum, internal capsule, and subcortical areas compared to control participants (Lipton et al., 2008). These areas are similar to those implicated at the acute recovery phase.

Niogi et al. (2008a) showed FA reductions in several white matter regions in symptomatic patients who were 1-65 months post-injury. In the patients with negative clinical MRI scans, slower reaction time was correlated with the number of white matter regions shown to have low FA values. A follow-up study with overlapping participants showed low FA in the uncinate fasciculus was correlated with worse memory performance, while low FA in the left anterior corona radiata was correlated with worse attentional capacity (Niogi, et al., 2008b). At an average of three years post injury, Wada, Asano, and Shinoda (2012) found that those with a medically diagnosed mTBI with lasting post-concussive symptoms had decreased FA values in the superior longitudinal fasciculus, superior frontal gyrus, insula, and fornix compared to nonconcussed controls. Cognitive functioning was positively correlated with FA in the mTBI group. These results show that white matter integrity may be a useful biomarker for cognitive dysfunction in mTBI.

A recent meta-analysis that included acute and chronic mTBI studies examined specific regions of the brain (Aoki et al., 2012). They found reduced FA and increased MD in the corpus callosum with no evidence of publication bias and with minimal heterogeneity across empirical studies using a random effects model. This effect appeared to be driven by aberrant values in the posterior, rather than anterior, portions of the corpus callosum. However, there was no evidence of differences in the mTBI group in the internal capsule or the corona radiata (Aoki, et al., 2012).

It is important to recognize the methodological differences across these DTI studies. They occurred at different intervals after injury with players potentially experiencing different types of concussions (e.g. location of impact, loss of consciousness vs. not, negative MRI findings vs. not, symptomatic vs. not). They also occurred on MRI scanners of different strengths (i.e. 1.5 and 3 Tesla) and were collected from different DTI scanning protocols. Further, they were analyzed in different ways (e.g. whole-brain analysis, region of interest based analysis) across different regions of the brain with different dependent variables (e.g. FA, MD). However, the convergence among the results is striking given the differences in methodology (Shenton et al., 2012).

These changes have also been seen in athletes who play contact sports but who have not experienced a concussion. In a study of NCAA division III football players, there were significant pre-season to post-season changes to FA and MD compared to non-athlete controls (Bazarian et al., 2014). These white matter differences persisted to a third MRI scanning session, which took place 6-months after no-contact rest. The amount of FA change was associated with the number of sub-concussive impacts as detected by helmet-mounted accelerometers (Bazarian et al. 2014). Similar results were indicated in a DTI study using high school football players, such that in the absence of an mTBI, the number of head impacts was linearly associated with all

of their DTI indices (Davenport et al., 2014). There was also a strong correlation between DTI measures and changes in verbal memory score pre- to post-season. Professional soccer players without concussions were reported to have increased RD and AD in several brain regions typically associated with mTBI when compared to swimmers (Koerte et al., 2012). In a cohort of amateur soccer players, self-reported frequency heading a soccer ball over the past year was associated with lower FA in the temporo-occipital cortex (Lipton, et al., 2013). Worse memory score was associated with lower FA, while past concussive history was not associated with either FA or cognitive performance. Another study showed that non-concussed collegiate contact sport athletes differed on MD compared to non-contact sport athletes (McAllister et al., 2014). Head impact exposure was associated with MD across varies brain regions, including the corpus callosum and hippocampus. Further, changes in MD were associated with worse memory functioning.

Multiple concussions

Literature suggests that those with a history of mTBI are at four to six times greater risk for having a second concussion (Guskiewicz et al., 2003). Most commonly, athletes will sustain a second concussion during the same season as their first concussion, suggesting that one's threshold for sustaining a concussion may be lowered during the acute phase of recovery (Guskiewicz et al., 2003). Guskiewicz et al. (2007) reported a positive correlation between the number of past concussions and the likelihood of being diagnosed with clinical depression. Additionally, obtaining a closed head injury has also been associated with an increased risk of developing Alzheimer's disease (Plassman et al., 2000; Schofield et al., 1997) as well as clinically diagnosed Mild Cognitive Impairment (MCI) and self-reported memory impairment (Guskiewicz et al., 2005). Further, Alzheimer's disease has been shown to onset earlier in life in

professional football players with a history of multiple mTBIs compared to the general American male population (Guskiewicz et al., 2005).

Receiving multiple concussive injuries is also associated with the development of dementia pugilistica, or chronic traumatic encephalopathy (CTE). Most common in former athletes (e.g. retired boxers and football players), CTE typically manifests after retirement from sports, most commonly in one's 40s (McKee et al., 2009). Initial symptoms include deterioration in attention, concentration, and memory, as well as increases in confusion, and sometimes dizziness and headaches (Millspaugh, 1937). Over time, overt dementia and several other symptoms begin to manifest which include impeded speech, abnormal gait, social instability, and symptoms of Parkinson's disease. All confirmed cases of CTE have been found in people who have sustained multiple concussions throughout the course of their life, over 90% of whom were athletes (McKee et al., 2009). However, this does not mean that all persons with several TBIs will get CTE. It is estimated that in the populations that sustain multiple concussions, such as chronic boxers, 17% later develop CTE. The precise incidence is unknown but may be even higher (Roberts, Allsop, & Bruton, 1990).

Multiple concussions may also have cognitive effects that signify brain pathology. History of at least two concussions has been associated with poorer performance on tests measuring executive functioning and processing speed (Collins et al., 1999; Gardner, Shores, & Batchelor, 2010), attention and concentration (Moser, Schatz, & Jordan, 2005), verbal memory and reaction time (Covassin, Moran, & Wilhelm, 2013), and overall neuropsychological functioning (Moser & Schatz, 2002). Athletes with two concussions in the same season showed declines in visuomotor speed, decreased visual learning, and increased errors on visual processing tasks (Pedersen, Ferraro, Himle, Schultz, & Poolman, 2014). Middle-aged athletes

with multiple concussions sustained in early adulthood had lower performance on tests of visual episodic memory and selective attention/ executive functioning/ response inhibition, providing evidence for chronicity of cognitive changes related to concussion (De Beaumont et al., 2009). Subtle cognitive effects of post-acute multiple concussions in young adults have been shown at liberal statistical thresholds (Terry et al., 2012). However, other studies have failed to find differences between athletes who sustained multiple concussions and non-concussed controls (Gaetz, Goodman, & Weinberg, 2000; Iverson, Brooks, Collins, & Lovell, 2006; Iverson, Brooks, Lovell, & Collins, 2006; Macciocchi, Barth, Littlefield, & Cantu, 2001).

To date, only one meta-analysis has been conducted on people with multiple concussions. Belanger, Spiegel, & Vanderploeg (2010) examined a total of 614 multiple mTBI cases and 926 one mTBI control cases using varied neuropsychological tests from multiple cognitive domains. Each of the included studies examined an average of 3.6 cognitive domains (range 1-6). The overall effect of multiple concussions on neuropsychological functioning was minimal and not significant when analyzing the overall effect sizes of the ten studies (d = 0.06). However, the Q statistic was statistically significant, suggesting heterogeneity of effect sizes across studies. When using cognitive domain as a categorical moderator, it was found that the multiple concussion group exhibited lower scores on measures of executive functioning (d = .24) and delayed memory (d = .16).

Given the association between multiple head injuries and later neurodegenerative conditions, it is especially important to use imaging modalities to try to detect brain abnormalities in those who have experienced multiple concussions. Brain pathology is the true diagnostic feature of neurodegenerative diseases like Alzheimer's disease and CTE. However, these syndromes cannot currently be diagnosed using a laboratory test, but rather are defined by

cognitive, clinical, and functional symptoms. Confirmation of these diseases cannot be confirmed until brain examination at autopsy. Having novel brain imaging metrics serve as biomarkers for disease processes will help delineate the biological and pathophysiological changes that occur as individuals become cognitively impaired. Such biomarkers have already been identified for Alzheimer's disease (Jack et al., 2011; Sperling et al., 2011), but none related to the potential for CTE.

Present Study

We extended the current literature with our examination of former high school football players who had at least two mild TBIs related to their high school football experiences. However, these former athletes have not received a concussion since that time, a period of at least fifteen years. We compared the concussed group to a matched group of control participants who also played high school football but do not have a history of concussive injuries. This is the first study to our knowledge to examine structural brain differences in former high school athletes who experienced multiple concussions. We were also interested as to how these injuries may be related to worse cognitive performance.

This study had two aims. The first used high-resolution FSPGR MRI scans from a 3-Tesla imaging system to elucidate volumetric differences between the past-concussed group and the control group. Previous reports have showed that those with an mTBI have higher rates of overall brain atrophy, white matter atrophy, and ventricular enlargement (Hofman, et al., 2001; Gale et al., 1995; MacKenzie et al., 2002) as well as atrophy in specific regions of interest (Singh et al., 2014; Monti, et al., 2013; Tate et al., 2014). We compared the two groups on total white matter volume, total brain volume, hippocampal volume, anterior cingulate volume, and

ventricular size, with the expectation that the mTBI group would have smaller volumes across all measures of brain volume but larger ventricles.

The second aim was related to white matter integrity. We examined whether different cognitive processes as measured on neuropsychological tests are sensitive to white matter degeneration occurring in mTBI. Based on the previous literature and the cognitive impairments associated with diseases like Alzheimer's and CTE, we expected FA, AD, and RD to be lower in the mTBI group compared to the control group on a whole-brain analysis, while MD will be higher in the mTBI group. We also expected correlations between *a priori* white matter tracts (i.e. corpus callosum, uncinate fasciculus, and anterior limb of the internal capsule) and cognitive indices due to the impact of white matter degeneration on neuropsychological functioning. Specifically, we expected 1) Diffusion indices FA and AD for the uncinate fasciculus would positively correlate with the RBANS Delayed Memory Score and Attention Score, while MD and RD would negatively correlate; 2) Diffusion indices FA and AD for the corpus callosum would positively correlate with the Total RBANS Score while MD and RD would negatively correlate; 3) Diffusion indices FA and AD for the anterior limb of the internal capsule would positively correlate with the Total RBANS Score while MD and RD would negatively correlate.

CHAPTER 2

METHOD

Participants

Power Analysis

To determine the necessary sample size for this study, means and standard deviations were extracted from aspirational empirical studies examining the differences between people with mTBIs and control participants on both volumetric MRI and DTI measures (i.e. Monti et al., 2012; Lipton et al., 2009; Singh et al., 2014). These metrics were converted to Cohen's *d* using the formula, $d = (M_1\text{-}M_2)/s_{pooled}$, where $s_{pooled} = \sqrt{[(s_1^2 + s_2^2)/2]}$. This resulted in one *d* value for each study. We chose the most conservative *d* value (i.e. the smallest value) of 0.95 for our power analysis. G*Power 3 (Faul et al., 2007) was used to calculate the appropriate sample sizes for the groups. Parameters for G*Power were: a-priori analysis, t-tests for independent means (two groups), two-tailed, $\alpha = 0.05$, and power $(1 - \beta) = 0.80$. The power analysis calculated that a sample size of nineteen would be needed for each group to achieve the statistical power necessary to detect the differences we expect to see. This value is consistent with the greater literature in this field. Given the funding allowance, we collected imaging data for twenty participants in each group.

Recruitment/Exclusionary Criteria

Data collection for this study began in 2012 as part of a larger dataset. Potential study participants responded to newspaper advertisements, online advertisements, and news articles about the study, or were contacted by researchers via email or phone based on information

gained though public records and football alumni listservs. Participants were included if they were right-handed, male, and aged 40-65 years. This age range was selected to maximize the number of participants who may have sustained a concussion in their remote history, but limit the frequency of people who by virtue of their age may be experiencing symptoms of MCI or dementia as this would be a confounding factor. Participants were excluded if they: were incompatible with the MRI environment (e.g., aneurism clip, pacemaker, cochlear implant, metallic stent, electronic implant), younger than 40 years, older than 65 years, reported being illiterate, reported being left-handed, reported learning English as a second language, have a history of alcohol or drug abuse/dependency within the past five years, reported a history of significant neurological disorder (e.g., seizures, epilepsy), reported a history of a developmental learning disorder (e.g. learning disability, ADD, ADHD), reported current use of any psychotropic medications, or reported a diagnosis of bipolar disorder or schizophrenia. One hundred and forty five potential participants from the surrounding Athens area were contacted regarding participation (see Figure 2.1). Of those, 45 participants were recruited and consented. Of these participants, imaging data is only available for 40 participants (i.e. n=20 in the mTBI group and n=20 in the control group). Upon completion, participants were given $50 for their participation.

Participants were divided into two groups: one group with a history of two or greater concussions in the context of their high school football experiences with no other lifetime concussions (total n=25; imaging n=20); and one group without any concussive history (total n=20; imaging n=20). Concussions were identified through the completion of a self-report questionnaire regarding concussive history aimed at validating the presence of at least two lifetime concussions based on criteria set by the American Congress of Rehabilitation Medicine

(Medicine, 1993), where a mTBI was diagnosed when at least one of the following criteria was met after an injury involving the head: (1) any period of loss of consciousness; (2) any loss of memory for events immediately before or after the accident; (3) any alteration in mental state at the time of the accident (e.g. feeling dazed, disoriented or confused) and (4) focal neurological deficit(s) that may or may not be transient (Medicine, 1993; Cassidy et al., 2004). Participants were also administered the Acute Concussion Evaluation (ACE; Giola & Collins, 2006), a systematic evidence-based clinical protocol designed to assess 1) the specific characteristic of the injury including the details of the blow to the head, 2) a full array of 22 symptoms and 5 signs associated with mTBI, and 3) risk factors that might predict a prolonged recovery. The ACE has been shown to have moderate to high internal consistency (α=0.82) and adequate content, predictive, and convergent validity when compared to other concussion assessments (Giola, Collins, & Isquith, 2008). Other studies using retrospective reporting showed adequate recall of remote historical facts with high one-month test-retest reliability (Kendler, Jacobson, Myers, & Eaves, 2007; Moffitt, et al., 2007). The groups were matched on age [$t(38) = 1.31$, $p = 0.20$], education [$t(38) = 0.59$, $p = 0.56$], and pre-morbid IQ level [$t(38) = 0.49$, $p = 0.63$] based on independent samples t-test analyses.

Materials & Procedure

This study was a portion of a larger investigation that conducted in the University of Georgia Neuropsychological and Memory Assessment Laboratory. As a result, these measures are within an extensive behavioral and neuroimaging protocol. This investigation only represents a portion of the measures and procedures that are completed by included participants. Potential participants were screened through an initial telephone call to assess their eligibility for the study. If eligibility criteria were met, they were scheduled for an appointment. At the beginning

of the study visit, participants sign a University of Georgia Institutional Review Board approved informed consent document. Participants were told participation is voluntary and that the study could be discontinued at any time.

Participants completed a concussion symptom checklist to index current post-concussive symptoms, if any. The Symptom Assessment Scale (SAS) is a 22-item symptom list where the participant ranks both the duration and the severity of each system over the past 24 hours using a Likert scale (0 to 6); the maximum total score is 132 on each of the two sub-scales (Broglio, Macciocchi, & Ferrara, 2007). The severity sub-scale is anchored with *not severe at all* and *as severe as possible,* and the duration scale is anchored with *briefly* and *always*.

Green's Medical Symptom Validity Test (Green, 2004) was administered to assess for adequate task engagement. This short, computerized verbal memory test was used to assess each participant's memory and symptom validity based on their level of performance on each trial and consistency in responding over trials. Participants were first presented with a set of ten semantically associated word-pairs across two trails (e.g. SHOE–LACE). They were then given an immediate recognition test during which each word from the original list was paired with a new semantically-related word (e.g. SHOE or SOLE). Participants indicated which of the two words appeared on the original list via a button press. After a ten-minute delay interval, participants were given a second recognition test in which each word from the original list was paired with another new associate word (e.g. SHOE or BOOT). Participants must have achieved a score considered to show effortful participation (85%) to continue with the study. Examination of participants' MSVT score profiles has shown to effectively discriminate people with dementia and people suspected of malingering from cognitively normal people without financial incentives (Howe et al., 2007). All participants met or exceeded this threshold.

The neuropsychological battery included the Wechsler Test of Adult Reading (WTAR), a 50-item word reading test that estimates pre-morbid intellectual ability by incorporating participants' performance with demographic variables (i.e., age, gender, education, ethnicity; Green et al., 2008). This well-validated measure in which participants pronounce words of increasing difficulty is being used to control for the potential confound of premorbid intellectual functioning differences between the concussed and control group.

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used to assess overall cognitive functioning (Randolph, Tierney, Mohr, & Chase, 1998). This battery of tasks is well validated for samples with possible neuropsychological deficits. It consists of 12 subtests and takes approximately 30 minutes to complete. These data yield 5 Index Scores across the cognitive domains Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory functioning as well as one Overall Scaled Score. The RBANS was standardized on a sample of 540 adults divided into six age groups (e.g. 20-29; 30-39, 40-49, etc.). Sex and race/ethnicity for each sample age group were proportionate to the levels indicated by the U.S. Census report of 1995. Education level was divided into three groups (i.e. less than high school, high school graduate, more than high school). The average reliability coefficient for each index across age groups was: Total=.94, immediate memory=.88, visuospatial/constructional=.80, attention=.85, language=.82, delayed memory=.83. Convergent and divergent construct validity was established using patient groups. Comparisons between various well know neuropsychological tests demonstrated the RBANS correlated highly with most of these test, showing convergent validity (Randolph, 1998).

Imaging Parameters

All images were acquired using a General Electric (GE; Waukesha, WI) Signa HDx 3T MRI. This scanner is equipped with 16 RF receiver channels with TQ Engine gradients (amplitude, 45 mT/m [z-axis], 40 mT/m [x, y-axes]; slew rate, 200 T/m/s) and an 8-channel head coil. 3D structural scans were acquired using a fast spoiled gradient recalled echo (FSPGR) protocol; TE </= 2ms, TR = 7.5 ms, flip angle = 20°, 154 axial slices, slice thickness = 1 mm, and FOV = 256 x 256 mm. These images cover from the top of the head to the brainstem. Acquisition for this sequence took approximately 6 min 20 s.

Diffusion weighted imaging (DWIs) scans were acquired axially using a single-shot diffusion-weighted SE-EPI sequence along the anterior commissure-posterior commissure line delineated from the FSPGR. Scan parameters include TE </= 2ms, TR = 15000 ms, 90° flip angle, 60 interleaved slices, slice gap = 0 mm, 2 mm isotropic voxels, acquisition matrix = 128 x 128, FOV = 256 x 256 mm, parallel acceleration factor = 2, b-value: 1000, and 30 optimized gradient directions with three b0 images. Total scan time for the DTI acquisition was 9 min and 38 s. To unwarp the DW images, we acquired two pairs of magnitude and phase images; TE1 = 5.0 ms and TE2 = 7.2ms, TR = 750 ms, 60 slices, slice gap = 0 mm, 2mm isotropic voxels, acquisition matrix = 128 x 128, and FOV = 256 x 256 mm. Acquisition for each pair of images took approximately 2 min 20 s. Additionally, several other scan sequences were collected on the participants, but were not included in the current analysis. Total scanning time was approximately 55 minutes.

Data Analysis

Volumetric MRI analysis

Automated segmentation of cortical and subcortical regions was conducted using Freesurfer (v 5.1). Details of these segmentation processes are available in Fishel et al. (2002; 2004a). In brief, this volume-based stream was designed to preprocess MRI volumes and label subcortical tissue classes. It consists of five preprocessing stages, the first of which registered the MRI scan to Talairach space using a basic affine transformation. This step is designed to be insensitive to pathology and to maximize the accuracy of the final segmentation. Then, anatomical labels were initially applied to the image. Next, the variation in intensity due to the B1 bias field was corrected. Finally, a high dimensional nonlinear volumetric alignment to the Talairach atlas was performed.

After the preprocessing, the volume was labeled based on both a subject-independent probabilistic atlas and subject-specific measured values. To accurately label these structures, the Freesurfer atlas was utilized. This atlas was built from a training set, i.e., a set of subjects whose brains have been labeled by hand. These labels were then mapped into a common space (i.e. Talairach space for volumes and spherical space for surfaces) to achieve voxel-to-voxel correspondence for all subjects. At each voxel in space, there exists the label that was assigned to each subject and the measured value for each subject. Labeling anatomical structures in a new data set relies on finding the segmentation that maximizes the probability of input given the prior probabilities from the training set. An iterative process of computing and recomputing the probability that a certain voxel belongs to a specific anatomical label takes place until the results are stable. This procedure allows the atlas to be customized for each data set by using the information specific to that data set.

This automated method has been shown to be statistically indistinguishable from the manual tracing of cortical and subcortical structures (Fischl, et al., 2002) and has been shown to be sensitive to volumetric differences between groups (Morey et al., 2009). Additionally, it is relatively insensitive to changes in acquisition parameters (Fishel, et al., 2004b), making this procedure robust and generalizable across different research settings. This method allowed for the extraction of the size (i.e. volume) for the following *a priori* regions of interest (ROIs): total intracranial volume (ICV), total gray matter, total white matter, left/right hippocampus, left/right cingulate cortex (anterior).

An automated measure of total intracranial volume (ICV) was also used in conjunction with the Freesurfer package (Buckner et al., 2004). Extracting ICV using this method has also been shown to be comparable to manual tracing. This volume served as both a dependent measure as well as a way to correct ROIs for overall head size. To control for ROIs, the slope (*b*) of the ROI volume was regressed onto the ICV. That variable was entered into the following equation to normalize each ROI for head size:

[normalized ROI volume = raw ROI volume – *b* (ICV – mean ICV)] [Formula 1]

This correction procedure has been used in multiple studies that have extracted subcortical volumes (Erickson, et al., 2009, Head, et al., 2008).

Between-groups independent samples t-test were conducted at a significance threshold of $p < .01$, which is corrected to control for multiple comparisons. The following t-tests were run between the concussed group and control groups: 1) whole brain volume, 2) normalized white matter volume, 3) normalized gray matter volume, 4) normalized bilateral hippocampal volume, 5), normalized bilateral anterior cingulate volume.

Diffusion Tensor Imaging Analysis

DWI images were preprocessed using the Oxford Centre's FMRIB Diffusion Toolbox (FDT; Behrens, et al., 2003). This toolbox allows for the correction of eddy currents motion during the scan using the first b0 image as a reference, given that eddy currents induce stretches and shears in the diffusion weighted images. It also extracted the brain using BET and corrected distortion using the calculated fieldmaps. Diffusion tensors were estimated for each voxel using DTIFIT (see Figure 2.2 for flowchart).

Tract-Based Spatial Statistics (TBSS; Smith et al., 2006), part of FSL (Smith et al., 2004) was utilized to examine differences in white matter integrity between the mTBI and control group. This method has been shown to be robust as it does not rely on the arbitrary nature of the registration of multiple participants' scans into a common space and the extent of spatial smoothing. Instead, it uses an individually based nonlinear registration and an alignment-invariant tract representation (e.g. the mean FA skeleton). First, TBSS determined which participant's data is most representative of the entire sample and then affine-aligned it into standard space. Then, all other participants' images were transformed into standard space by combining the nonlinear transform to the target FA image (FNIRT, using a b-spline representation of the registration warp field; Rueckert 1999) with the affine transform from that target to standard space (Andersson, et al., 2007a, 2007b). Next, the mean FA image were created and thinned to make a mean FA skeleton, which represents the centers of all tracts common to the group. Each subject's aligned FA data were then projected onto this skeleton and the resulting data were fed into voxelwise cross-subject statistics (Figure 2.2).

The two groups were compared using FSL's Randomise function, which analyzed the skeletonized data in a nonparametric, permutation-based way given that the null distribution for

the data is unknown (Winkler et al., 2014). Output was computed based on a threshold-free cluster-enhancement (TFCE) framework, which enhances detection of diffusivity differences by using neighborhood information to determine the statistical significance of a cluster of voxels instead of making the user set an unnecessarily conservative threshold at the outset that could lead to a Type II error. For each instance of Randomise, 5000 null permutations were generated to test against the data. The two groups were compared using t-statistics at a TFCE-based *p*-value of .05 for the following indices: FA, MD, AD, RD.

To examine the association between the integrity of *a priori* white matter bundles (i.e. corpus callosum, uncinate fasciculus, anterior limb of the internal capsule) and cognition, we used fiber tractography methods using 3D Slicer software (www.slicer.org). This method helps to ensure precise segmentation of the white matter structures by relying on anatomically-driven manual selection of regions of interest (ROIs) on DTI scans. The multiple ROI method has been used before in several clinical tractography studies (e.g., Catani et al., 2002; Jones et al., 2005a,b). We used the fiber assignment by continuous tracking (FACT) method to extract the fiber tract, which makes fiber trajectories from seeding points (Mori et al., 1999). This method initiates fiber trajectories and follows the primary eigenvector from voxel to voxel. Tractography stopping criteria were set to be: FA < 0.15 and an angle of curvature > 20° per 1 mm (Jones et al., 2006).

Regions of interest were drawn on the DTI scan based on previous literature. For the anterior limb of the internal capsule (ALIC), we followed the procedures outlined in Levitt et al. (2010), which spans approximately four coronal slices. In this paper, they validated a method defining the anterior, posterior, medial, lateral superior, and inferior boundaries of the structure (see Appendix A). Yasmin et al. (2008) developed a method for drawing two ROIs for reliable

tractography of the uncinate fasiculus. The corpus callosum was defined by drawing ROIs as discussed by Huang et al. (2005) and Lebel, Caverhill-Godkewitsch, & Beaulieu (2010). The results of the tractography were converted ("voxelized") into binary maps to allow the extraction of DTI data. FA, RD, AD, and MD were calculated for each tract in each person.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS version 18.0). Correlations between these three tracts and RBANS neuropsychological scores were made. Specifically, we examined correlations between the overall RBANS score and all four DTI indices for the corpus callosum and the ALIC. For the uncinate fasciculus, we examined the relationship between the overall RBANS score, attention score, and memory score. A statistical threshold of $p < .01$ was used to control for the multiple comparisons.

Figure 2.1 Flow chart of recruitment

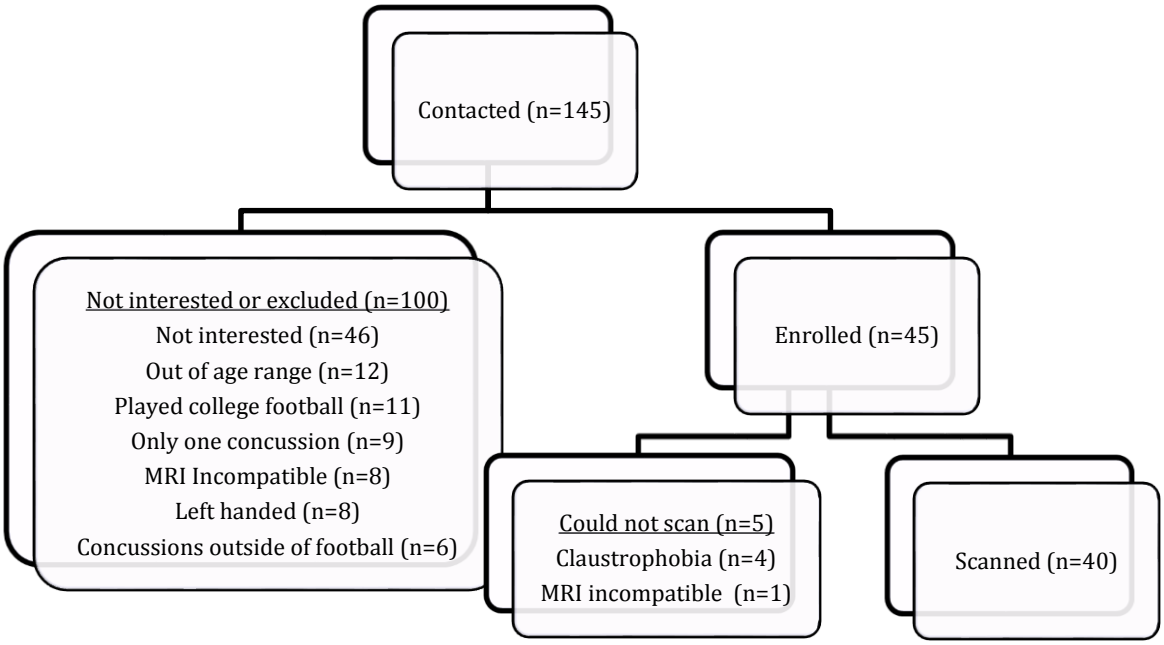
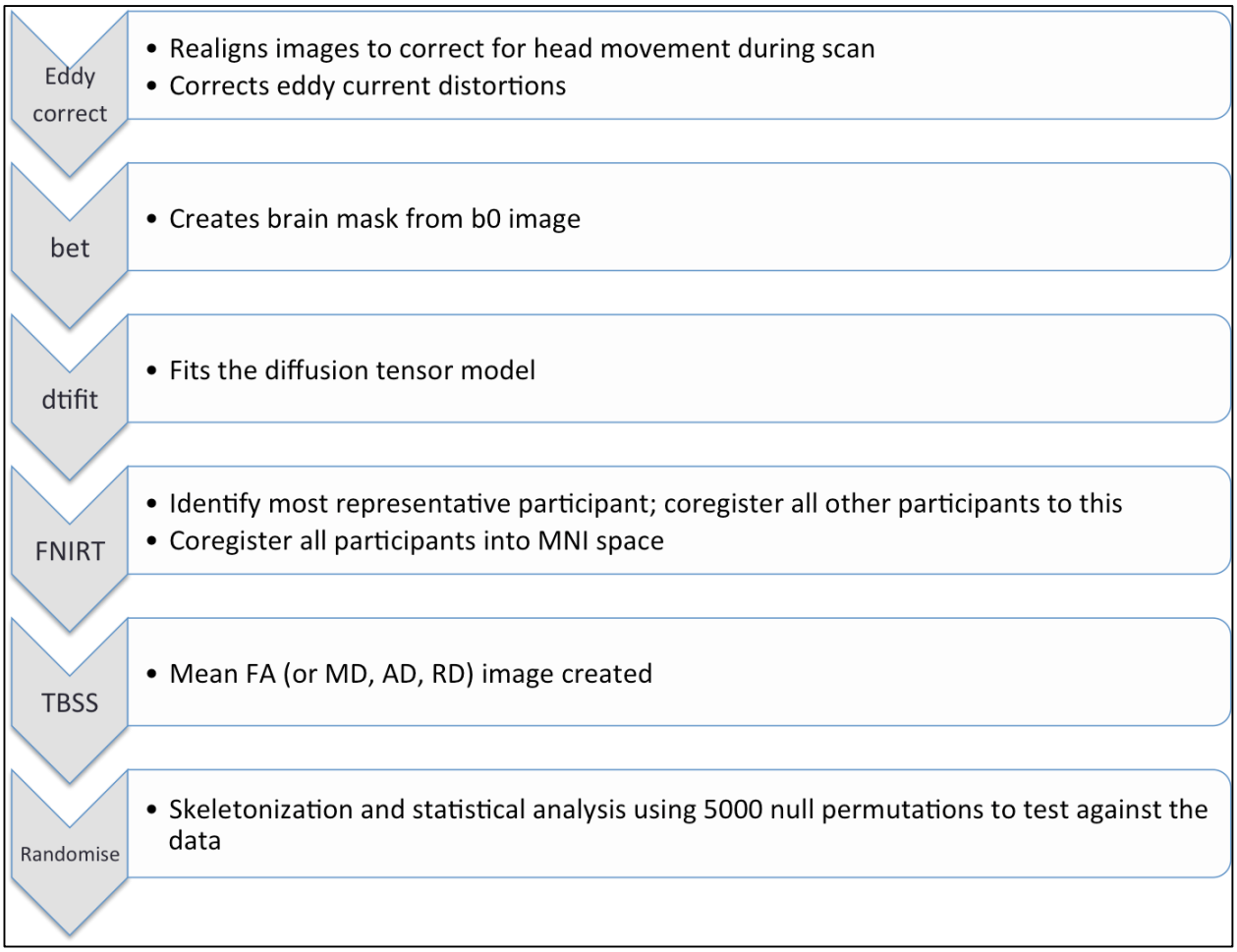


Figure 2.2 Diffusion Tensor Imaging Pipeline



CHAPTER 3

REPEATED MILD TRAUMAUTIC BRAIN INJURIES NOT ASSOCIATED WITH VOLUMETRIC DIFFERENCES IN FORMER HIGH SCHOOL FOOTBALL PLAYERS [1]

[1] Terry, D. P., & Miller, L. S. To be submitted to the *Brain, Imaging, and Behavior*.

Abstract

The chronic neuroanatomical effects of mild traumatic brain injuries (mTBIs) are currently being assessed in several populations, as the characteristics of the injury (e.g. loss of consciousness, neuroimaging findings, number of mTBIs) appear to be associated with study results. We investigated these potential volumetric differences in a sample of former high school football players many years after these injuries. Forty community-dwelling males ages 40-65 who played high school football, but not college or professional sports, were recruited. The experimental group (n=20) endorsed experiencing two or more mTBIs on an empirically validated mTBI assessment tool (median=3, range=2-15). The control group (n=20) denied ever experiencing an mTBI. Participants completed a self-report index of current mTBI symptomatology and underwent high-resolution T1-weighted MRI scanning, which were analyzed using the Freesurfer software package. *A priori* regions of interest (ROIs) included total intracranial volume (ICV), total gray matter, total white matter, bilateral anterior cingulate cortex, and bilateral hippocampi. ROIs were corrected for head size using a normalization method that takes ICV into account. Despite being well matched on age, education, estimated premorbid IQ, and current concussive symptomatology, there were no statistically significant volumetric group differences across all of the ROIs despite a sample size larger than in previous studies with positive findings. These data suggest that multiple mTBIs from high school football may not be associated with measurable brain atrophy later in life. Accounting for the severity of injury and chronicity of sport exposure may be especially important when measuring long-term neuroanatomical differences.

Introduction and Literature Review

Mild traumatic brain injuries (mTBI) are becoming increasingly common, as over 75% of the 1.7 million traumatic brain injuries in the United States each year are classified as mild using a Glascow Coma Scale score between 13 and 15 (CDCP, 2010; Faul 2010). The symptoms of an mTBI are clinically heterogeneous, typically manifesting as a variety of cognitive (e.g. reduced attention and memory), somatic (e.g. headaches, dizziness, nausea), affective (e.g. sad, emotionally labile), and sleep-related difficulties (CDCP, 2010; McCrory et al., 2009; Arciniegas et al., 2000; Hall, Hall, & Chapman, 2005). However, accurate diagnosis of an mTBI can be difficult due to the lack of radiological evidence of a brain injury, as the brain is frequently deemed to be normal on traditional brain imaging modalities such as computerized tomography (CT) and magnetic resonance imaging (MRI) (e.g., Bazarian et al. 2007; Hughes et al. 2004, Iverson et al., 2000). Thus, the diagnosis of an mTBI is typically based on self-reported clinical and cognitive symptoms following head trauma. This can be problematic as the symptoms of an mTBI are nonspecific and can occur in a variety of other medical and psychological conditions (Stein and McAllister, 2009).

However, there may be ways to improve neuroimaging methods to more sensitively detect brain abnormalities sustained related to an mTBI. There is evidence that there are, in fact, pathophysiological changes associated with an mTBI, as autopsy studies have suggested that some who have experienced an mTBI with negative radiological findings have microscopic axonal injuries (Bigler, 2004; Blumbergs et al., 1994). These changes are likely the result of the mechanical impact of the head injury. Rapid acceleration and deceleration forces related to the concussive event are thought to stretch, twist, and shear axons or cause alterations in cell function that manifest as the acute symptoms of an mTBI (Blennow, Hardy, and Zutterberg,

2012). Magnetic resonance imaging (MRI) scans show promise in detecting these abnormalities, as they are more sensitive in detecting white matter shearing, small subacute hemorrhages, small contusions, and small foci of axonal injury as compared to CT (see Niogi and Mukherjee, 2010). MRI can also better differentiate brain and CSF as well as edema as compared to CT scans (Johnston, et al., 2001), which are important considerations when evaluating mTBI using imaging modalities.

Many studies have shown chronic (i.e. greater than six months post injury) differences in brain volume following a concussion, such as decreased overall brain volume (Gale et al., 1995; MacKenzie et al. 2002). Further, these studies suggested that loss of consciousness and worse neurocognitive functioning was associated with smaller brain volumes in the patient groups. This conclusion was upheld in a study examining symptomatic patients with an mTBI at an average of two years post-injury, which showed that the mTBI patients had a higher rate of atrophy in forebrain parenchyma, cerebral white matter, and cerebellar volume as compared to controls at two different time points (Ross et al., 2012). Tate et al. (2014) examined cortical thickness on a sample of veterans who experienced a blast-related mTBI within 18-months of injury and found that the mTBI group exhibited cortical thinning in the left superior temporal and left superior frontal gyri, which were associated with abnormal language and audition scores on behavioral measures.

Longitudinal studies of neuroanatomical changes in mTBI have produced mixed findings. A group of people with a documented mTBI in an emergency department setting exhibited global brain atrophy compared to a control group on longitudinal scans taken acutely after the mTBI and again at 1-year post-injury (Zhou et al., 2013). In collegiate hockey players, global volume reductions were noted after a concussion at 2-weeks and 2-months after a concussion

(Jarrett et al., 2016). Region of interest analyses indicated reduced gray matter volume in the precuneus and white matter volume in the anterior cingulate, which correlated with memory and attention performance (Zhou et al., 2013). Hofman et al., (2001) showed that when scanned again at six months post-injury, patients with abnormal MRIs had a greater rate of brain atrophy than those without MRI abnormalities. In another study, the patient group had abnormally rapid atrophy in whole brain parenchyma and cerebral white matter between the time of the injury and an average of 1.7 years later, while the cerebellum and brainstem enlarged rapidly over the first year and a half and then diminished over the following year, suggesting that different brain regions are affected by a TBI in different ways (Ross et al., 2014). However, in a study examining ventricular volume, gray matter volume, and cortical thickness during the acute (i.e. within three weeks) and semi-acute phase of recovery (i.e. four months later), there were no significant differences between the mTBI group and a matched control group (Ling et al., 2013).

Looking at specific regions within the brain has also showed differences associated with mTBI. Monti et al. (2013) found that those who experienced a medically diagnosed mTBI before the age of 25 years old had smaller bilateral hippocampi compared to well-matched control participants at an average of 30 years post-injury. They also found that this sample did worse on a face-scene relational memory task and had less functional MRI activity in memory-related regions in the parietal and prefrontal cortices. However, these differences were not evident in younger samples after an average of four years post-injury (Monti, et al., 2013), suggesting that these subcortical changes may take place over a longer amount of time.

Many of the studies presented thus far have been conducted in medical populations, where a concussion may occur at one discrete time point. However, athletes that play contact sports may have different brain changes in response to a concussion due to the accumulation of

chronic impact to the head. In a sample of current college football players, participants that experienced a clinician-diagnosed mTBI had smaller bilateral hippocampi compared to a sample of non-concussed teammates as well as a sample of non-concussed non-athletes at a post-injury period ranging from 1 day to 4.5 years (Singh, et al., 2014). Additionally, the non-concussed football players had smaller bilateral hippocampi compared to the control group, suggesting that the sub-concussive impact associated with football may have an impact on brain structure. The time players reported engaging in football was negatively correlated with hippocampal volume suggestive of a dose-response relationship (Singh et al., 2014).

More chronic changes have also been documented in sports-related mTBI. Cortical thinning of the right temporal lobe and left insula was seen in a sample of young adult athletes with multiple concussions who had not had a concussion in the past 6-months as compared to non-concussed controls (List, Ott, Burkowski, Lindenberg, & Floel, 2015). In a sample of former college athletes ages 50-75 who experienced between one and five concussions (average = 2.08) before their mid-20s, authors found cortical thinning in frontal, temporal, and parietal areas when comparing the previously concussed athletes to their non-concussed teammates (Tremblay et al., 2013). Authors also found abnormal enlargement of the lateral ventricles and worse memory performance for the injured group, which resemble patterns of abnormal aging reflected in neurodegenerative conditions often seen later in life.

We extended the current literature with our examination of former high school football players who had at least two mild TBIs related to their high school football experiences. However, these former athletes have not received a concussion since that time, a period of at least fifteen years. We compared the concussed group to a matched group of control participants who also played high school football but do not have a history of concussive injuries. This is the

first study to our knowledge to examine structural brain volumes in former high school athletes who experienced multiple concussions. We also examined how these injuries may be related to worse cognitive performance. We used high-resolution FSPGR MRI scans from a 3-Tesla imaging system to elucidate volumetric differences between the past-concussed group and the control group with *a priori* hypotheses that the past-concussion group would have less total brain volume (i.e. intracranial volume, ICV), total white matter volume, total gray matter volume, hippocampal volume, and anterior cingulate volume compared to the control group. Further, explored the relationship between hippocampal volume with memory functioning, as well the between gray matter volume and overall cognitive functioning.

Method

Participants

Data collection for this study began in 2012 as part of a larger dataset. Preliminary functional MRI data were previously published on a subset of these participants (Terry, Adams, Ferrara, & Miller, 2015). Recruitment took place through newspaper and online advertisements, news articles about the study, or public records and football alumni listservs. Participants were included if they were right-handed, male, and aged 40-65 years. This age range was selected to maximize the number of participants who may have sustained a concussion in their remote history, but limit the frequency of people who by virtue of their age may be experiencing symptoms of MCI or dementia as this would be a confounding factor. Participants were excluded if they reported: being incompatible with the MRI environment, being illiterate, learning English as a second language, a history of alcohol or drug abuse/dependency within the past five years, a history of a neurological disorder (e.g., seizures, epilepsy), a history of a developmental learning disorder (e.g. learning disability, ADD, ADHD), current use of any psychotropic medications, or

a diagnosis of bipolar disorder or schizophrenia. One hundred and forty-five potential participants were screened, from which 45 were successfully recruited and consented by signing an Institutional Review Board approved consent form. Of these participants, imaging data is only available for 40 participants (i.e. n=20 for each group) due to claustrophobia and late-identified MRI incompatibility. Participants were given a small stipend ($50) for their participation.

Participants were divided into two groups: one with a history of two or greater concussions in the context of their high school football experiences but no other lifetime concussions (total n=25; imaging n=20); and one without any concussive history (total n=20; imaging n=20). Concussions were identified through the completion of a self-report questionnaire regarding concussive history aimed at validating the presence of at least two lifetime concussions based on criteria set by the American Congress of Rehabilitation Medicine (Medicine, 1993), where a mTBI was diagnosed when at least one of the following criteria was met after an injury involving the head: (1) any period of loss of consciousness; (2) any loss of memory for events immediately before or after the accident; (3) any alteration in mental state at the time of the accident (e.g. feeling dazed, disoriented or confused) and (4) focal neurological deficit(s) that may or may not be transient (Medicine, 1993; Cassidy et al., 2004). Participants were also administered the Acute Concussion Evaluation (ACE; Giola & Collins, 2006), a systematic evidence-based clinical protocol designed to assess 1) the specific characteristic of the injury including the details of the blow to the head, 2) a full array of 22 symptoms and 5 signs associated with mTBI, and 3) risk factors that might predict a prolonged recovery. The ACE was shown to have moderate to high internal consistency (α=0.82) and adequate content, predictive, and convergent validity when compared to other concussion assessments (Giola, Collins, & Isquith, 2008). Previous studies using retrospective reporting showed adequate recall of remote

historical events with acceptable one-month test-retest reliability (Kendler, Jacobson, Myers, & Eaves, 2007; Moffitt, et al., 2007).

Participants also completed a concussion symptom checklist to index current post-concussive symptoms, if any, as this could have been a potential confound in the neuroimaging analysis. The Symptom Assessment Scale (SAS) is a 22-item symptom list where the participant first answers "yes" or "no" as to whether they have experienced any of the symptoms over the past 24 hours. For those symptoms positively endorsed, they rank both the duration and the severity of each system over the past 24 hours using a Likert scale (0 to 6); the maximum total score is 132 on each of the two sub-scales (Broglio, Macciocchi, & Ferrara, 2007). The severity sub-scale is anchored with *not severe at all* and *as severe as possible,* and the duration scale is anchored with *briefly* and *always*.

The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) was used to assess cognitive functioning (Randolph, Tierney, Mohr, & Chase, 1998). A total of 12 subtests are used to generate 5 index scores (i.e., Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory) and one Overall Scaled Score for the whole test. Raw scores are converted to standard scores (i.e., mean=100, standard deviation=15) based on age.

Neuroimaging

All images were acquired using a General Electric (GE; Waukesha, WI) Signa HDx 3T MRI. This scanner is equipped with 16 RF receiver channels with TQ Engine gradients (amplitude, 45 mT/m [z-axis], 40 mT/m [x, y-axes]; slew rate, 200 T/m/s) and an 8-channel head coil. 3D structural scans were acquired using a fast spoiled gradient recalled echo (FSPGR) protocol; TE </= 2ms, TR = 7.5 ms, flip angle = 20°, 154 axial slices, slice thickness = 1 mm,

and FOV = 256 x 256 mm. These images cover from the top of the head to the brainstem. Acquisition time for this sequence was 6 min 20 s. Total scanning time was approximately 55 minutes.

Volumetric MRI analysis

Automated segmentation of cortical and subcortical regions was conducted using Freesurfer (v 5.1). Details of these segmentation processes are available in Fischl et al. (2002; 2004a). This automated method has been shown to be statistically indistinguishable from the manual tracing of cortical and subcortical structures (Fischl, et al., 2002) and has been shown to be sensitive to volumetric differences between groups (Morey et al., 2009). Additionally, it is relatively insensitive to changes in acquisition parameters (Fischl, et al., 2004b), making this procedure robust and generalizable across different research settings.

An automated measure of total intracranial volume (ICV) was also used in conjunction with the Freesurfer package (Buckner et al., 2004). Extracting ICV using this method has also been shown to be comparable to manual tracing. This volume served as both a dependent measure as well as a way to correct ROIs for overall head size. To control for ROIs, the slope (*b*) of the ROI volume was regressed onto the ICV. That variable was entered into the following equation to normalize each ROI for head size:

[normalized ROI volume = raw ROI volume – *b* (ICV – mean ICV)]

This correction procedure has been used in multiple studies that have extracted subcortical volumes (Erickson, et al., 2009, Head, et al., 2008).

Behavioral data analysis.

Data were analyzed using the Statistical Package for Social Sciences (SPSS version 18.0). Between-groups independent samples t-test were conducted at a significance threshold of

$p < .01$, which is corrected to control for multiple comparisons. The following t-tests were run between the concussed group and control groups: 1) whole brain volume, 2) normalized white matter volume, 3) normalized gray matter, 4) normalized bilateral hippocampal volume, 5) normalized bilateral anterior cingulate volume.

Results

When comparing the previously concussed group with the non-concussed group, there were no statistically significant differences with respect to age [$t(38) = 1.31$, $p = 0.20$], years of formal education [$t(38) = 0.59$, $p = 0.56$], and pre-morbid IQ level as measured by the Wechsler Test of Adult Reading [$t(38) = 0.49$, $p = 0.63$] based on independent samples t-test analyses (Table 3.1). On the SAS, both participant groups reported experiencing a modest number of symptoms over the previous 24 hours (previously concussed group = 4.6; non-concussed group = 3.6). However, there were no group differences in the total number of symptoms reported [$t(38) = 0.85$, $p = 0.40$], the duration of those symptoms over the past 24 hours [$t(38) = 1.17$, $p = 0.25$], or the symptom severity [$t(38) = 1.49$, $p = 0.14$].

Independent t-test analysis comparing whole brain volume between the concussed and the control groups was not significant [$t(38) = 0.029$, $p = 0.98$] (Table 3.2). The average ICV for the sample was 1405323 mm^3, which was used to calculate the normalized volumes for each of the ROIs. There were no statistically significant differences in volumes between the two groups when comparing overall gray matter volume [$t(38) = 0.451$, $p = 0.65$], overall white matter volume [$t(38) = 0.432$, $p = 0.66$], bilateral anterior cingulate volume [$t(38) = 0.509$, $p = 0.61$], or bilateral hippocampal volume [$t(38) = 0.033$, $p = 0.74$].

In exploratory post-hoc analyses, we sought to examine if there was differential relationship between cognition and brain volumes. Using bivariate correlations, bilateral

hippocampal volumes were not correlated with the Delayed Memory index score on the RBANS for the overall sample [r = .05, p = .77], the concussed group [r = -.03, p = .90], or the control group [r = .12, p = .62]. However, better overall cognitive functioning was related to more adjusted gray matter volume in the entire sample [r = .48, p = .002] (Figure 3.1). This correlation appeared to be stronger in the concussed group [r = .53, p = .01] compared to the control group [r = .44, p = .06], though there was not a statistically significant difference when comparing the relationships between groups [z = 0.032, p = .37].

Discussion

Results of our study suggest a lack of volume differences in the brains of middle-aged men who experienced multiple mTBIs compared to those without mTBIs in the context of their high school football careers. This lack of volumetric differences was seen in both overall intracranial volume as well as in specific regions that have previously been associated with smaller volumes such as overall gray matter, white matter, the anterior cingulate, and the hippocampus.

It important to understand the sample recruited for this study in the context of its null results. The individuals in this study were community dwelling and actively recruited to take place in this research study based on local advertisements and historical records. This sample may be different than previous studies that recruited participants who sought professional medical attention or experienced loss of consciousness (e.g., Monti et al., 2013). Further, the mTBI group participants cumulatively reported only receiving medical attention after 31% of their concussions. Thus, our sample may represent more mild cases on the spectrum of what could be classified as an mTBI. Also unlike some previous studies, the mTBI group was not experiencing an increased number of concussion-like symptoms as compared to the non-mTBI

control group, nor were they labeled as having abnormal MRI findings from a clinical brain scan at the time of the injury (e.g., Hofman et al., 2001). Further, this sample assessed participants more remotely than many of the previous studies that reported positive findings (e.g., Singh, et al., 2014). Thus, it is possible that frank neuronal loss may only occur in the context of chronically symptomatic individuals or in the presence of positive imaging findings.

Statistical power to detect differences is important to consider in a study reporting a lack of statistically significant findings. An *a priori* power analysis calculated that a sample size of 38 (i.e. 19 per group) would achieve a power of .80 based on the effect sizes of previous empirical studies (e.g., Monti et al., 2013; Ross et al., 2012, Singh, et al., 2014) This value is consistent with the typical sample sizes of the literature in this field. There also do not appear to be any meaningful effect sizes when examining the means and standard deviations of our data. This suggests that the current results are likely not an artifact of low power to detect differences.

Lack of volumetric differences as suggested by these data does not mean this sample is free of neural-related differences. In a subsample (n=36) of these participants, we previously showed that the mTBI group exhibited less blood-oxygen-level dependent (BOLD) signal in left hemisphere brain regions associated with memory encoding and recognition during a verbal-paired associates task despite comparable task performance and memory scores on traditional neuropsychological tasks (Terry, Adams, Ferrara, & Miller, 2015). Additionally, recent analyses of diffusion tensor imaging data on this sample indicate reduced fractional anisotropy in the mTBI group in the right genu/body of the corpus callosum, bilateral internal capsule, bilateral superior longitudinal fasciculus, and bilateral infero-frontal longitudinal fasciculus (Terry & Miller, in preparation). Taken together, there appear to be some neuroanatomical abnormalities

in these participants, but they have not yet reached the level of gross brain atrophy or functional impairments in everyday life.

Exploratory post-hoc analyses did not show a relationship between hippocampal volume and memory functioning as would have been expected. However, they did show a positive relationship between overall cognitive functioning and gray matter volume as other studies have also shown between brain metrics and cognition (Tate, et al., 2014; Tremblay et al., 2013). This relationship did not differ between the concussed and control groups.

There are several limitations to this study. The heterogeneity of types of mTBI (e.g. severity, location of impact, loss of consciousness, etc.) limits the specificity of our results. Further, the lack of medical records related to the concussive events limits our ability to ensure the severity of the concussions. However, given the under-diagnosis of mTBIs due to historical views about mTBI, desire to continue to play, and other factors, we found that including subjects without professional medical attention would increase the external validity of the sample. Another limitation is the cross-sectional nature of this study, which limits our ability to conclude that group differences are the product of the mTBI events. Lastly, the current null findings may be an artifact of the time since the injury. Positive functional MRI and white matter findings but lack of gross volumetric differences may suggest that there is a pathogenic process occurring that could manifest as volumetric differences farther into the future.

Despite these limitations, this study adds to the overall body of literature related to potential neuroanatomic changes post-mTBI. We believe this is the first study to examine potential brain volume differences in former high school football players with and without multiple concussions. Data suggests there are no statistically significant differences in intracranial volume, white matter volume, or gray matter volume. Further, there was a lack of

group differences in more specific regions previously associated with mTBI (i.e. hippocampus, ACC). Future research is needed to assess whether volumetric differences manifest in these individuals later in life given the function imaging and diffusion tensor imaging findings.

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Table 3.1 Group Demographics

	Controls (n=20)	Concussed (n=20)	p-value
Age	50.0 (7.8)	53.1 (7.3)	.20
Years of Education	15.3 (2.0)	15.7 (2.2)	.56
WTAR	109.9 (11.6)	111.7 (11.8)	.63
Number of concussions	-	4.3 (3.7)	
Concussions with LOC	-	29.0%	
Concussions with medical attention	-	31.4%	
Concussions with memory lapse	-	46.5%	
SAS Number of symptoms	3.6 (2.7)	4.6 (4.8)	.40
SAS Average Sum Duration	8.7 (7.4)	13.7 (17.7)	.25
SAS Average Sum Severity	6.9 (6.0)	12.1 (14.3)	.14

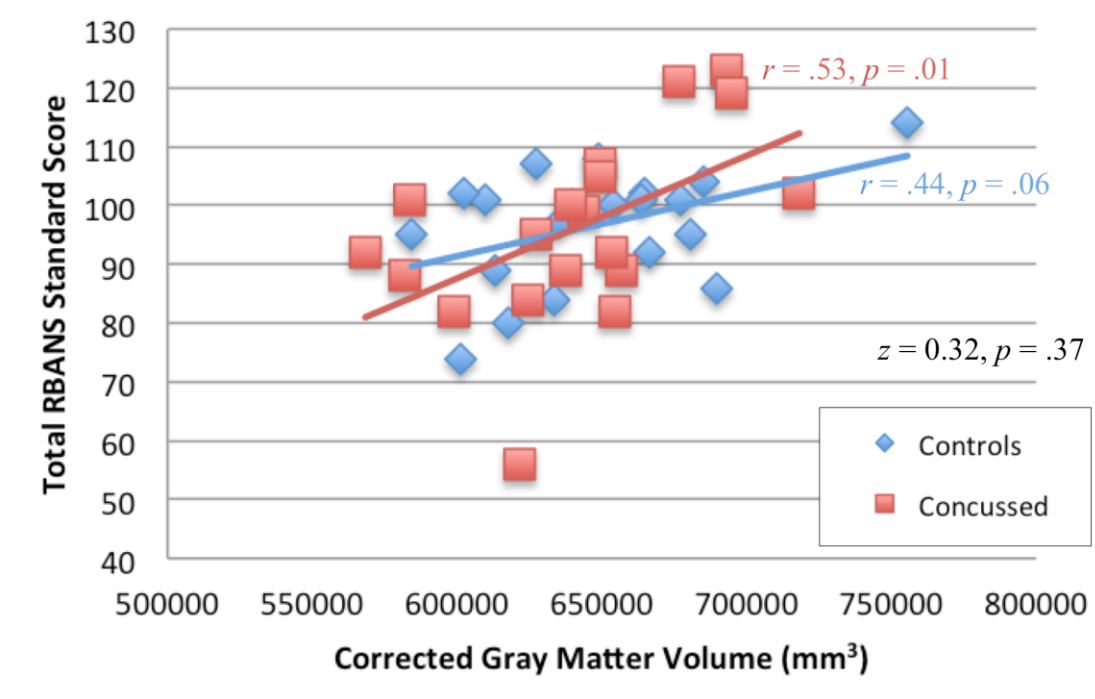
Note. Values are mean and (SD). The median number of concussions for the concussion group was 3. When two extreme values in the number of reported concussions were reduced to two standard deviations above the mean, the average number of concussions was 3.9. WTAR = Wechsler Test of Adult Reading; LOC = Loss of consciousness; SAS = Symptom Assessment Scale.

Table 3.2 Between-group independent t-test comparisons of brain volumes.

	Controls (n=20)	Concussed (n=20)	*b*	t-statistic	p-value	Cohen's d
Total ICV	1403012(205607)	1401335 (150241)	-	0.029	.98	0.009
Normalized Gray Matter	647125 (40950)	641371 (38714)	0.239	0.451	.65	0.144
Normalized White Matter	508075 (30155)	514024 (52306)	0.137	0.432	.66	-0.139
Normalized ACC	2883 (354)	2808 (527)	0.000474	0.509	.61	0.167
Normalized Hippocampi	8946 (1511)	9080 (972)	0.00501	0.33	.74	-0.105

Note. Values are in mm3. ICV = Intracranial volume; ACC = anterior cingulate cortex

Figure 3.1. Bivariate correlations between overall gray matter volume and standard score on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS).



CHAPTER 4

MICROSTRUCTURAL WHITE MATTER DIFFERENCES IN FORMER HIGH SCHOOL FOOTBALL PLAYERS WITH A HISTORY OF MULTIPLE CONCUSSIONS [2]

[2] Terry, D. P. & Miller, L. S. To be submitted to the *Journal of Neurotrauma.*

Abstract

Multiple concussions sustained in youth may be associated with later-life brain changes and worse cognitive outcomes. Here, we examine the association between sustaining two or more concussions in the context of high school football and later-life white matter (WM) microstructure using diffusion tensor imaging (DTI). Forty, right-handed former high school football players currently ages 40-65 were divided into two groups: those who received two or more concussions in the context of high school football (n=20), and those who denied concussive-like events but were matched on age, education, and premorbid IQ (n=20). Participants underwent neurocognitive testing and a DTI scan on a 3 Tesla magnet. Tract-based Spatial Statistics yielded several areas of lower fractional anisotropy (i.e., worse overall WM integrity) in the concussed group at a threshold of $p < .05$ corrected for multiple comparisons. These areas included the corpus callosum, superior longitudinal fasciculus, and internal capsule. *A priori* region of interest analyses using tractography showed higher radial diffusivity in the corpus callosum of the concussion group compared to the control group ($p < .01$), suggestive of dysmyelination. However, there were no between-group differences in the uncinate fasciculus and the anterior limb of the internal capsule on any diffusion indices. Concussive histories were not associated with worse cognitive functioning, nor were there relationships between individuals' neuropsychological scores and DTI metrics. These results suggest there may be subtle microstructural white matter changes in the corpus callosum and other brain regions that have yet to manifest as a measurable functional deficit.

Introduction and Literature Review

Mild traumatic brain injuries are often diagnosed based on their transient neurological, cognitive, and clinical symptoms due to the lack of evidence of an injury on traditional magnetic resonance imaging or computerized tomography scans (Bazarian et al. 2007; Hughes et al. 2004, Iverson et al., 2000). Diffusion tensor imaging (DTI) is an advanced MRI technique that has showed promise in elucidating a biological basis for this injury as it can identify microstructural white matter changes caused by the shearing of axons due to the acceleration, deceleration, and rotational forces of a brain injury (Bigler, 2013).

DTI is based on the motion of water molecules in the brain. Pathology is quantified based on the restriction of water flow based on the tissue being measured. Its main indices are mean diffusivity (MD) and fractional anisotropy (FA). MD is an index of overall water diffusion within the region, where higher MD is thought to indicate decreased white matter integrity. FA represents the overall directionality of flow within an area and is used as an index of overall white matter health (Beaulieu et al., 1996). Lower FA is generally thought to reflect loss of white matter integrity that may reflect damage to myelin or axon membrane damage, or reduced axonal packing density, and/or reduced axonal coherence. Because MD and FA can be greatly affected in regions where there are crossing fibers (Alexander et al., 2007; Vos et al., 2012), other indices have been used as they may be more related to specific microstructural abnormalities. Axial diffusivity (AD) describes the principal direction of water flow and is thought to be related to axonal or changes in extra-axonal/extracellular space (Glenn et al., 2003; Beaulieu and Allen, 1994). In contrast, radial diffusivity (RD) represents the average of the second and third eigenvalues of the estimated DTI tensor and is thought to be associated with changes in myelination or glial cell morphology (Song et al., 2002, 2003, 2005).

Given that DTI is sensitive to axonal injuries characteristic in TBI, it has been used to identify subtle brain abnormalities in mTBI acutely after an injury. Studies have showed reductions in FA and increases in MD acutely after an mTBI in various regions, including corpus callosum and internal capsule (Arfanakis et al., 2002), corpus callosum, internal capsule, and centrum semiovale (Inglese et al., 2005). Lower FA and higher MD have been associated with worse cognition, especially executive functioning skills (Miles et al. 2008; Lipton et al. 2009). Kumar et al. (2009) showed reduced FA as well as increased RD in the corpus callosum in people with mild and moderate TBIs within two weeks of their injury. Similar differences have been found at the semi-acute phase of recovery. Cubon & Putukian (2011) showed increased MD in the left inferior and superior longitudinal fasciculi, fronto-occipital fasciculus, internal capsule, and thalamic radiations at one month post-injury. Longitudinal analysis of collegiate athletes with an mTBI indicated that higher RD and lower FA were evident at both two days and two months post-injury in right hemisphere areas including the superior/inferior longitudinal fasciculi and internal capsule (Dettwiler, et al., 2014).

Not all studies of this nature have showed lower FA. Zhang et al., (2010) did not find DTI differences in the corpus callosum on student-athletes at 30 days post-injury. Lange et al. (2011) similarly found no FA and MD differences in people 6-8 weeks post-mTBI who sought emergency department treatment compared to orthopedic injury controls. Further, a growing number of studies have cited increases in FA post-injury (Bazarian et al., 2007, Lipton et al., 2012), which may be indicative of cytotoxic edema due to axonal swelling and more restricted water flow, which may lead to a poor clinical outcome (Kou et al., 2010). Alternatively, lower FA may indicate vasogenic edema, which will likely resolve over time. Thus, these discrepancies are likely due to a host of differences in the characteristics of the injury (e.g. location of impact,

loss of consciousness vs. not, negative MRI findings vs. not, symptomatic vs. not), different scanner strengths and sequences, analysis strategies (e.g. whole-brain vs. region of interest based) across different regions of the brain with different dependent variables (e.g. FA, MD; Shenton et al., 2012).

Several investigators have examined the chronic changes associated with white matter integrity in mTBI and its relationship with cognition. Lower FA and higher MD were shown in the corpus callosum, internal capsule, and subcortical areas at eight months to three years post injury (Lipton et al., 2008), while lower FA and higher AD were shown in the corticospinal tract, superior longitudinal fasciculus, and sagittal striatum nine years post injury. Meta-analytic evidence of lower FA and higher MD in the corpus callosum has been supported with no evidence of publication bias and with minimal heterogeneity across empirical studies using a random effects model (Aoki, et al., 2012). Globally lower FA has been associated with slower reaction time (Niogi et al., 2008a), while lower FA in the unicinate fasciculus has been associated with worse memory performance and lower FA in the left anterior corona radiata was correlated with worse attentional capacity (Niogi, et al., 2008b). Lower FA has also been associated with lower scores on a brief cognitive screening and IQ scores in those with chronic post-concussive symptoms in multiple brain regions (Wada, Asano, and Shinoda, 2013).

White matter changes are also evident in contact sport athletes in the absence of a concussion. Collegiate football players showed significant pre-season to post-season changes in FA and MD compared to non-athlete controls that persisted to a third MRI scanning session that took place 6-months after no-contact rest (Bazarian et al. 2014). The amount of FA change has been associated with the number of sub-concussive impacts as detected by helmet-mounted accelerometers as well as memory ability (Bazarian et al. 2014; Davenport et al., 2014). Non-

concussed collegiate contact sport athletes have also differed on MD compared to non-contact sport athletes in various brain regions, including the corpus callosum and hippocampus (McAllister et al., 2014). Further, changes in MD were associated with worse memory functioning.

Using diffusion tensor imaging, we examined white matter integrity in a group of middle-aged former athletes who received at least two mTBIs in the context of their high school football experiences compared to their non-concussed teammates. Based on previous literature and the cognitive impairments associated with diseases like Alzheimer's and CTE, we expected FA and AD to be lower in the mTBI group compared to the control group on a whole-brain analysis, and expected MD and RD to be higher in the mTBI group. We also expected correlations between *a priori* white matter tracts (i.e. corpus callosum, uncinate fasciculus, and anterior limb of the internal capsule) and cognitive indices due to the impact of white matter degeneration on neuropsychological functioning. Specifically, we expected 1) Diffusion indices FA and AD for the uncinate fasciculus would positively correlate with the RBANS Delayed Memory Score and Attention Score, while MD and RD would negatively correlate; 2) Diffusion indices FA and AD for the corpus callosum would positively correlate with the Total RBANS Score while MD and RD would negatively correlate; 3) Diffusion indices FA and AD for the anterior limb of the internal capsule would positively correlate with the Total RBANS Score while MD and RD would negatively correlate.

Method

Participants

These data are a part of a larger dataset, for which other imaging modalities have already been published (i.e., Terry, Adams, Ferrara, & Miller, 2015). Participants were recruited through

newspaper and online advertisements, online new articles discussing the study, and football alumni listservs maintained through local coaches and athletic trainers. Participants were enrolled if they were right-handed, male, and between the ages of 40 and 65 and did not endorse any of the following exclusionary criteria: incompatibility with the MRI environment, history of substance abuse/dependence in the past five years, illiteracy, learning English as a second language, having a neurological disease, having a developmental learning disorder, a having a diagnosis of schizophrenia or bipolar disorder, or currently taking a psychotropic medication. Of the 145 individuals who were screened, 45 enrolled in the study by signing a consent form approved by the Institutional Review Board approved consent form. Five subjects did not undergo scanning due to claustrophobia and late-identified MRI incompatibilities. Participants were given small honoraria ($50) for their efforts.

Concussions were assessed using a two-step process. First, participants completed a self-report questionnaire designed for them to recall instances across their lifetime during which they received a head injury that met one or more of the following criteria set forth by the American Congress of Rehabilitation Medicine (1) any period of loss of consciousness; (2) any loss of memory for events immediately before or after the accident; (3) any alteration in mental state at the time of the accident (e.g. feeling dazed, disoriented or confused) and (4) focal neurological deficit(s) that may or may not be transient (Medicine, 1993; Cassidy et al., 2004). If two discrete head injuries were reported in the context of high school football, participants were administered the Acute Concussion Evaluation (ACE; Giola & Collins, 2006). This systematic, evidence-based interview assesses the specific characteristics surrounding of the injury, 22 symptoms and 5 signs associated with an mTBI, and risk factors that may predict a prolonged recovery. This semi-structured clinical interview has an internal consistency of α=0.82 and adequate content,

predictive, and convergent validity (Giola, Collins, & Isquith, 2008). Based on this process, participants were divided into one of two groups: those with a history of two or greater concussions in the context of high school football without any other lifetime concussive events (total n=25; imaging n=20) and those without any concussive events (total n=20; imaging n=20). Those with exactly one concussion and those with non-football related concussions were excluded from the study.

Measures

Wechsler Test of Adult Reading (WTAR). This 50-item word reading test estimates pre-morbid intellectual ability by incorporating participants' performance with demographic variables (Green et al., 2008) and was used to control for the potential confound of premorbid intellectual functioning differences between the concussed and control group.

Symptom Assessment Scale (SAS) is a 22-item symptom list where the participant reflects on the previous 24 hours and identifies 1) the presence or absence of each concussion-like symptoms, 2) the duration of the symptom on a 0-6 Likert scale anchored with *briefly* and *always*, and 3) the severity of the symptom on a 0-6 Likert scale anchored with *not severe at all* to *as severe as possible* (Broglio, Macciocchi, & Ferrara, 2007).

Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) assessed overall cognitive functioning with twelve subtests, which yielded five Index Scores (i.e. Visuospatial/Constructional, Attention, Language, Immediate Memory, and Delayed Memory) and one Overall Scaled Score (Randolph, Tierney, Mohr, & Chase, 1998). Adequate reliability and convergent/divergent construct validity have been well established using patient groups (Randolph, 1998).

Green's Medical Symptom Validity Test (Green, 2004) is a short, computerized verbal memory test used to assess for adequate task engagement. Participants must have achieved a score considered to show effortful participation (85%) to continue with the study as this score has effectively discriminated people with dementia and people suspected of malingering from cognitively normal people without financial incentives (Howe et al., 2007). All participants (n=45; 100%) met or exceeded this threshold.

Neuroimaging

Images were acquired using a General Electric (GE; Waukesha, WI) Signa HDx 3T MRI scanner, which is equipped with 16 RF receiver channels with TQ Engine gradients (amplitude, 45 mT/m [z-axis], 40 mT/m [x, y-axes]; slew rate, 200 T/m/s) and an 8-channel head coil. Diffusion weighted imaging (DWIs) scans were acquired axially using a single-shot diffusion-weighted SE-EPI sequence along the anterior commissure-posterior commissure line delineated from the FSPGR. Scan parameters include TE </= 2ms, TR = 15000 ms, 90° flip angle, 60 interleaved slices, slice gap = 0 mm, 2 mm isotropic voxels, acquisition matrix = 128 x 128, FOV = 256 x 256 mm, parallel acceleration factor = 2, b-value: 1000, and 30 optimized gradient directions with three b0 images. Total scan time for the DTI acquisition was 9 min and 38 s. To unwarp the DW images, we acquired a pair of magnitude and phase images; TE1 = 5.0 ms and TE2 = 7.2ms, TR = 750 ms, 60 slices, slice gap = 0 mm, 2mm isotropic voxels, acquisition matrix = 128 x 128, and FOV = 256 x 256 mm. Acquisition for the pair of images took approximately 2 min 20 s. Additionally, several other scan sequences were collected on the participants, but are not included in the current analysis.

DTI analysis

DWI images were preprocessed using the Oxford Centre's FMRIB Diffusion Toolbox (FDT; Behrens, et al., 2003), which corrects for eddy currents and motion during the scan, extracts the brain, and corrects distortion using the calculated fieldmaps. Diffusion tensors were estimated for each voxel using DTIFIT. Tract-Based Spatial Statistics (TBSS; Smith et al., 2006), part of FSL (Smith et al., 2004) was utilized to examine differences in white matter integrity between the mTBI and control group. First, TBSS determined which participant's data is most representative of the entire sample and then affine-aligned it into standard space. Then, all other participants' images were transformed into standard space by combining the nonlinear transform to the target FA image (FNIRT, using a b-spline representation of the registration warp field; Rueckert 1999) with the affine transform from that target to standard space (Andersson, et al., 2007a, 2007b). Next, the mean FA image was created and thinned to make a mean FA skeleton, which represented the centers of all tracts common to the group. Each subject's aligned FA data was then projected onto this skeleton and the resulting data was fed into voxelwise cross-subject statistics.

The two groups were compared using FSL's Randomise function, which analyzed the skeletonized data in a nonparametric, permutation-based way given that the null distribution for the data is unknown (Winkler et al., 2014). Output was viewed from a threshold-free cluster-enhancement (TFCE) framework, which enhances detection of diffusivity differences by using neighborhood information to determine the statistical significance of a cluster of voxels instead of making the user set an unnecessarily conservative threshold at the outset that could lead to a Type II error. For each instance of Randomise, 5000 null permutations were generated to test

against the data. The two groups were compared using t-statistics at a TFCE-based *p*-value of .05 for the following indices: FA, MD, AD, RD.

To examine the association between the integrity of *a priori* white matter bundles (i.e. corpus callosum, uncinate fasciculus, anterior limb of the internal capsule) and cognition, we used fiber tractography methods using 3D Slicer software (www.slicer.org; Surgical Planning Laboratory, Brigham & Women's Hospital, Boston MA). This method helps to ensure precise segmentation of the white matter structures by relying on anatomically-driven manual selection of regions of interest (ROIs) on DTI scans. The multiple ROI method has been used before in multiple clinical tractography studies (e.g., Catani et al., 2002; Jones et al., 2005a,b). We used the fiber assignment by continuous tracking (FACT) method to extract the fiber tract, which makes fiber trajectories from seeding points (Mori et al., 1999). This method initiates fiber trajectories and follows the primary eigenvector from voxel to voxel. Tractography stopping criteria were: FA < 0.15 and an angle of curvature > 20° per 1 mm (Jones et al., 2006). All cases were drawn blind to group status. The results of the tractography were converted ("voxelized") into binary maps to allow the extraction of DTI data. Average FA, RD, AD, and MD were calculated for each tract in each participant for the 1) bilateral uncinate fasciculus, 2) bilateral anterior limb of the internal capsule, and 3) corpus callosum.

For the anterior limb of the internal capsule (ALIC), we followed the procedures outlined in Levitt et al. (2010), which spans approximately four coronal slices. In this paper, they validate a method defining the anterior, posterior, medial, lateral superior, and inferior boundaries of the ALIC by outlining the caudate, putamen, lateral ventricle CSF, and ventral striatum. This leaves a central region that represents the ALIC, from which we started the tractography seeding.

Yasmin et al. (2008) developed a method for drawing two-ROI for reliable tractography of the uncinate fasiculus (UF). The first ROI was placed at the anterior UF on a coronal slice at the anterior portion of the genu of the corpus callosum, directly anterior to the horn of the lateral ventricle. The posterior ROI was placed on a coronal slice at the most anterior portion of the temporal stem. Only fibers that passed through both ROIs were included as part of the UF.

The corpus callosum was defined manually by finding the midsagittal slice on a color-oriented FA map. Similar to the procedures of Huang et al. (2005) and Lebel, Caverhill-Godkewitsch, & Beaulieu (2010), we manually outlined the corpus callosum on the midsagittal slice and its adjacent slices. To ensure only corpus callosum fibers were included, we used exclusionary ROIs on axial slices near the thalamus and midbrain to remove corticothalamic and corticospinal fibers. Extraneous fibers were removed manually by the lead author on a case-by-case basis.

Behavioral data analysis.

Data were analyzed using the Statistical Package for Social Sciences (SPSS version 18.0). For the tractography regions of interest, each participant's DTI metrics were compared using between-groups independent samples t-test at a significance threshold of $p < .01$, which is corrected to control for multiple comparisons. Hypothesized relationships between region of interest tractography values and individual cognition scores (i.e., RBANS Total score, RBANS Attention score, and RBANS delayed memory score) were calculated using bivariate Pearson correlations at a significance threshold of $p < .01$.

Results

There were no statistically significant differences between the previously concussed and non-concussed groups with respect to age [$t(39) = 1.01$, $p = 0.28$], years of formal education [

t(39) = 0.28, *p* = 0.78], and pre-morbid IQ level as measured by the Wechsler Test of Adult Reading [*t*(39) = -0.63 , *p* = 0.53] based on independent samples t-test analyses (Table 4.1). Further, at the time of scanning, the two groups reported similar levels of concussion-like symptoms on the Symptom Assessment Scale (see Table 4.1). Further, there were no statistically significant between-group differences on neuropsychological variables (Table 4.2).

Results of the TBSS white matter analysis yielded several clusters of voxels with statistically significant FA values when comparing the concussed and non-concussed groups. The concussed group had a lower FA than the control group in seven TCFE-determined clusters. The minimum of the largest cluster (17717 voxels) was in the right hemisphere but the statistically significant regions spanned several white matter tracts bilaterally. These tracts include the genu and body of the corpus callosum, the inferior fronto-longitudinal fasciculus, internal capsule, superior longitudinal fasciculus, and fornix. Parts of these tracts were also shown to have statistically lower FA values in the left hemisphere (see Table 4.2; Figure 4.1). There were no regions of lower FA in the control group as compared to the concussed group.

Other diffusion metrics were also compared using TBSS (i.e., MD, AD, and RD). However, none of these metrics yielded statistically significant results in either direction (see Table 4.3).

Diffusion indices were also compared between groups across the corpus callosum, ALIC, and UF. P values were adjusted to $\leq .01$ in an *a priori* manner given the number of comparisons. In the corpus callosum (Figure 4.2), the concussed group had a higher RD compared to the control group [*t*(39) = 3.6143, *p* = .0009]. Fractional anisotropy appeared to be lower in the concussed group, but did not reach the point of statistical significance [*t*(39) = 2.6996, *p* = .0103]. A similar pattern showed potentially higher MD and lower AD in the concussed group

compared to the control group (Table 4.4). There were no between-group differences for the UF and ALIC on any diffusion metrics. Further, there were no statistically significant correlations between diffusion indices and cognitive variables (Table 4.5).

Discussion

This study is the first to evaluate the relationship between multiple mTBI history during high school football and later-life white matter microstructure in a cohort of middle-aged, former high school football players. Using a whole-brain methodology, we observed lower FA in the concussion group across a variety of white matter tracts compared to a well-matched group of former football players who denied ever receiving a concussion-like injury. However, there were no group differences using other diffusion indices. Using a more hypothesis-driven approach, we examined the corpus callosum, bilateral uncinate fasciculus (UF), and bilateral anterior limb of the internal capsule (ALIC). Tractography revealed higher RD in the corpus callosum, with other indices that approached statistical significance. There were no group differences for the UF and ALIC, nor were any of the diffusion metrics associated with hypothesized cognitive variables.

Whole-brain TBSS showed widespread differences in regard to lower FA in the concussed group. These differences occurred in the anatomical locations associated with several white matter tracts, such as the genu of the corpus callosum (i.e., the anterior portion), the splenium of the corpus callosum (i.e., the posterior portion), the internal capsule, the superior longitudinal fasciculus, inferior fronto-longitudinal fasciculus, and uncinate fasciculus, among others. It is important to note that these locations are generated based on a framework that groups spatially similar (i.e., neighboring) voxels irrespective of neuroanatomical delineations. The minimum cluster size and significance threshold is determined by the data itself, making it an atheoretical exploration of the entire brain. Results were as expected regarding lower FA in the

concussed group; however, this is less telling about the type of potential cellular-level changes that are present given that FA has been shown to be sensitive to many types of cell changes (e.g., cell death, edema, gliosis, changes in intracellular/extracellular fluid, changes in myelination, crossing fibers) but non-specific to any individual change (O'Donnell & Pasternak, 2015). In the absence of significant findings on other diffusion metrics (i.e., AD, RD, and MD), we are currently unable to hypothesize about the potential microstructural changes associated with a history of concussions with the TBSS data.

Region of interest analyses using tractography methodology may elucidate more specific changes white matter changes in the corpus callosum. Radial diffusivity was statistically higher in participants with at least two previous mTBIs compared to control participants. Higher RD has reliably shown to be indicative of less myelination using rodent models (Song, 2002) and has recently been implicated as being higher in former professional football players who started playing tackle football before the age of 12 as compared to their teammates that started to tackle at age 12 or older (Stamm, et al., 2015). The authors of this study divided the corpus callosum into five distinct anatomical regions based on a mathematical scheme linked to the functional projections of the fibers as validated in Hofer & Frahm (2006). Stamm et al. (2015) found lower FA and higher RD in only the anterior portions of the corpus callosum, but did not find these differences when examining the corpus callosum as a whole. They posited that the repetitive head impacts associated with tackle football, combined with exposure to such forces at a time that is critical for the myelination of the corpus callosum, may potentially lead to a disruption of normal myelination processes and subsequently lead to a lower peak level of myelination as adults. Although we did not ask the participants of this study the age at which they began to tackle, it is worth considering this hypothesis given the consistency of these results and that it is

likely that many of the current study's participants starting playing tackle football at an early age. However, there are theoretical investigations that caution the use of RD in predicting myelin pathology (Wheeler-Kingshott & Cercignani, 2009), so further research is also needed to understand the clinical implications of higher RD.

It is worth commenting on the potential reasons why whole-brain TBSS detected differences in white matter integrity in the UF and ALIC but tractography did not. From an intraindividual perspective, MD has relatively small variability within different parts of white matter, while FA, AD, and RD are highly spatially variable. For instance, FA is high at tract centers and drops abruptly at tract edges and in areas of crossing fibers. Methodologically, TBSS analyzes only the high FA data at the center of the tract (i.e., the "skeleton") and does not analyze the peripheral, low FA parts of the white matter tracts. It also functions independent of anatomical delineations, and thus, may only denote a small part of the entire white matter tract as being statistically different. Thus, the results of the tractography may not be statistically significant because of the inclusion of many low FA voxels that could dilute the between-group differences. Given that it is currently unclear as to which of these methods is more clinically relevant, (O'Donnell & Pasternak, 2015), we find these methodological differences important to discuss as they sample different parts of the white matter structure.

These results extend previous work suggesting subtle neuroanatomical changes in the brains of former high school football players. Terry and colleagues previously found that former high school football players had left-hemisphere hypoactivation during a verbal learning and memory task using functional magnetic resonance imaging, despite a lack of behavioral differences on the learning paradigm or learning/memory differences between the two groups on traditional neurocognitive tests (Terry et al., 2015). List and colleagues showed cortical thinning

in the right temporal lobe and left insula a cohort of athletes that received at least two concussions at a minimum of 6-months post injury in the absence of differences between groups on neuropsychology test scores (List et al., 2015). These data also suggest anatomical brain changes in former athletes, but a lack of neuropsychological test differences. Taken together, these results suggest modest yet chronic structural changes that as a result of multiple head injuries may be sustained during the critical period of adolescent development.

Limitations, Strengths, and Future Directions

There are several caveats and strengths in this study to take into account. We only examined high school football players who received their mTBIs in the context of this sport. These results may not be generalizable to other sports (i.e., soccer, hockey) or to civilian/military injuries due to the different mechanisms of action and rotational factors associated with each. Further, this study may not be generalizable to female athletes given the notable differences between the genders in the recovery from a concussion (Zuckerman, Apple, Odom, Solomon, & Sills, 2014). Also, the cross-sectional nature of this study limits our ability to associate early mTBIs with brain changes in a causal manner, though the groups were well matched on many variables known to be associated with white matter changes (i.e., age, sex, education, current concussive symptomatology). In this study, concussions were measured via self-report in a retrospective manner given most concussions were never reported to medical staff, and even if they were, those records likely are not available at present. Although an examiner confirmed group membership using a validated concussion interview (i.e., the ACE), which is more rigorous than many other studies, participants' memory ability and nonspecific factors (e.g., personality style, knowledge about concussions) could have influenced their reporting. This study also employed an index of effort/task engagement (i.e., the MSVT) in case of exaggerated

cognitive complaints. All participants performed adequately on this task, and thus, the cognitive results are thought to represent their true neurocognitive ability.

Future studies should employ prospective methodologies to track adolescents who sustain mTBIs in a longitudinal fashion to help determine causal relationships and have a better-matched control group. Further, future studies will need to account for the impact of subconcussive blows. In the current study, we were only interested in the added impact of frank concussions, but both groups in this study were subject to repetitive head trauma related to their participation in tackle football. A non-contact sport control group should be recruited for studies that want to examine the effect of repetitive brain trauma. Related to our findings in the corpus callsoum, we plan to divide this structure into its five functional regions based on the previously outlined criteria to examine if a specific sub-region is driving the current effect.

Conclusions

This study found that former high school football players that endorsed two or more concussions in the context of high school football had lower FA in several brain regions as identified by whole-brain examination as compared to non-concussed former football players, as well as a higher RD in the corpus callosum upon region of interest analysis using tractography methodology. There were no differences in cognitive variables, nor were there associations between white matter indices and cognitive performance. These results suggest subtle neuroanatomical changes in the brains of athletes potentially related to altered myelin micropathology. Future research is needed to delineate potential neurodevelopmental compared to neurodegenerative changes as a result of concussive injuries.

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Table 4.1 Group Demographics.

	Controls (n=20)	Concussed (n=20)	p-value
Age	50.0 (7.8)	53.1 (7.3)	.20
Years of Education	15.3 (2.0)	15.7 (2.2)	.56
WTAR	109.9 (11.6)	111.7 (11.8)	.63
Number of concussions	-	4.3 (3.7)	
Concussions with LOC	-	29.0%	
Concussions with medical attention	-	31.4%	
Concussions with memory lapse	-	46.5%	
SAS Number of symptoms	3.6 (2.7)	4.6 (4.8)	.40
SAS Average Sum Duration	8.7 (7.4)	13.7 (17.7)	.25
SAS Average Sum Severity	6.9 (6.0)	12.1 (14.3)	.14

Note. Values are mean and (SD). The median number of concussions for the concussion group was 3. When two extreme values in the number of reported concussions were reduced to two standard deviations above the mean, the average number of concussions was 3.9. WTAR = Wechsler Test of Adult Reading; LOC = Loss of consciousness; SAS = Symptom Assessment Scale.

Table. 4.2 Means, standard deviations, and independent samples t-test of index scores on the Repeatable Battery for the Assessment of Neuropsychological Status.

	Controls	Concussed	t-statistic	p-value
RBANS Immediate Memory	100.20 (13.83)	99.05 (18.42)	0.2233	.8245
RBANS Visuospatial	98.85 (10.09)	96.85 (16.92)	0.4540	.6524
RBANS Language	94.45 (7.97)	96.90 (10.75)	0.8097	.4233
RBANS Attention	102.20 (14.21)	102.30 (16.70)	0.0204	.9839
RBANS Delayed Memory	94.50 (12.21)	91.95 (14.31)	0.6061	.5480
RBANS Total Score	96.60 (9.94)	96.70 (15.74)	0.0201	.9841

Note. RBANS=Repeatable Battery for the Assessment of Neuropsychological Status.

Table 4.3. Tract-Based Spatial Statistics (TBSS) whole-brain analysis of diffusion indices between the previously concussed group and the control group at $p < .05$.

# Voxels	p-value	X	Y	Z	L/R	
						FA - Concussed < Control
17717	.02	17	-75	-5	R	genu CC, body of CC, inferior fronto-longitudinal fasciculus, IC, SLF, fornix
4919	.034	27	10	39	R	SLF, uncinate fasciculus
3083	.04	-31	-34	8	L	inferior fronto-longitudinal fasciculus , internal capsule , SLF
178	.045	27	-16	20	R	superior corona radiata, SLF, corticospinal tract
124	.046	-56	-38	8	L	SLF
19	.046	26	-13	32	R	superior corona radiata
13	.047	-13	-88	-11	L	inferior fronto-longitudinal fasciculus
9	.047	34	-33	22	R	SLF
2	.048	28	-20	26	R	superior corona radiata
						FA - Control < Concussed
						no suprathreshold clusters
						MD - Concussed < Control
						no suprathreshold clusters
						MD - Control < Concussed
						no suprathreshold clusters
						AD - Concussed < Control
						no suprathreshold clusters
						AD - Control < Concussed
						no suprathreshold clusters
						RD - Concussed < Control
						no suprathreshold clusters
						RD - Control < Concussed
						no suprathreshold clusters

Note. FA=fractional anisotropy. CC=corpus callosum. SLF=superior longitudinal fasciculus. IC=internal capsule. MD=mean diffusivity.

Table 4.4. Results of independent samples t-tests across regions of interest using diffusion tensor imaging metrics.

	Concussed (n=20)		Control (n=20)				
	Mean	SD	Mean	SD	t statistic	p value	Cohen's d
Whole CC							
FA	0.5432	0.02262	0.5626	0.02283	2.6996	.0103	-0.853
MD	0.000902	0.00005	0.000871	0.0000426	2.1106	.0414	0.667
AD	0.001524	0.0001465	0.001637	0.0001667	2.2771	.0285	-0.720
RD	0.000541	0.0000417	0.000489	0.0000490	3.6143	.0009	-1.143
Uncinate Fasciculus							
FA	0.4531	0.02377	0.4661	0.0239	1.7248	.0927	-0.545
MD	0.000883	0.0000488	0.000881	0.0000416	0.01395	.8898	0.044
AD	0.001411	0.0001323	0.00147	0.0001402	1.3688	.1791	-0.432
RD	0.000579	0.0000436	0.000589	0.0000403	0.7532	.4559	-0.238
Internal Capsule							
FA	0.4953	0.02244	0.4891	0.02295	0.8638	.3931	0.273
MD	0.000815	0.000047	0.000822	0.0000451	0.4806	.6336	-0.152
AD	0.001433	0.000159	0.00149	0.0001429	1.1924	.2405	-0.377
RD	0.000496	0.0000399	0.000479	0.0000417	1.3173	.1956	0.417

Note. FA=fractional anisotropy; MD=mean diffusivity; AD=axial diffusivity; RD=radial diffusivity

Table 4.5. Bivariate correlations between diffusion tensor indices and cognitive variables.

	Pearson r	p-value
Corpus Callosum	Total RBANS	
FA	.237	.141
MD	-.073	.654
AD	.090	.580
RD	.214	.185
ALIC	Total RBANS	
FA	-.159	.327
MD	-.221	.170
AD	-.020	.902
RD	.045	.783
UF	Delayed Memory	
FA	.077	.623
MD	-.299	.061
AD	-.107	.511
RD	.038	.816
UF	Attention	
FA	.182	.261
MD	-.225	.163
AD	.051	.755
RD	.193	.233

Note. ALIC = anterior limb of the internal capsule; UF = uncinate fasciculus; RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; FA = fractional anisotropy; MD = mean diffusivity; AD = axial diffusivity; RD = radial diffusivity

Figure 4.1 Tract-based spatial statistics results at Montreal Neurological Institute coordinates [-5, -16, 25] for fractional anisotropy for the [control group – concussed group] contrast at $p < .05$.

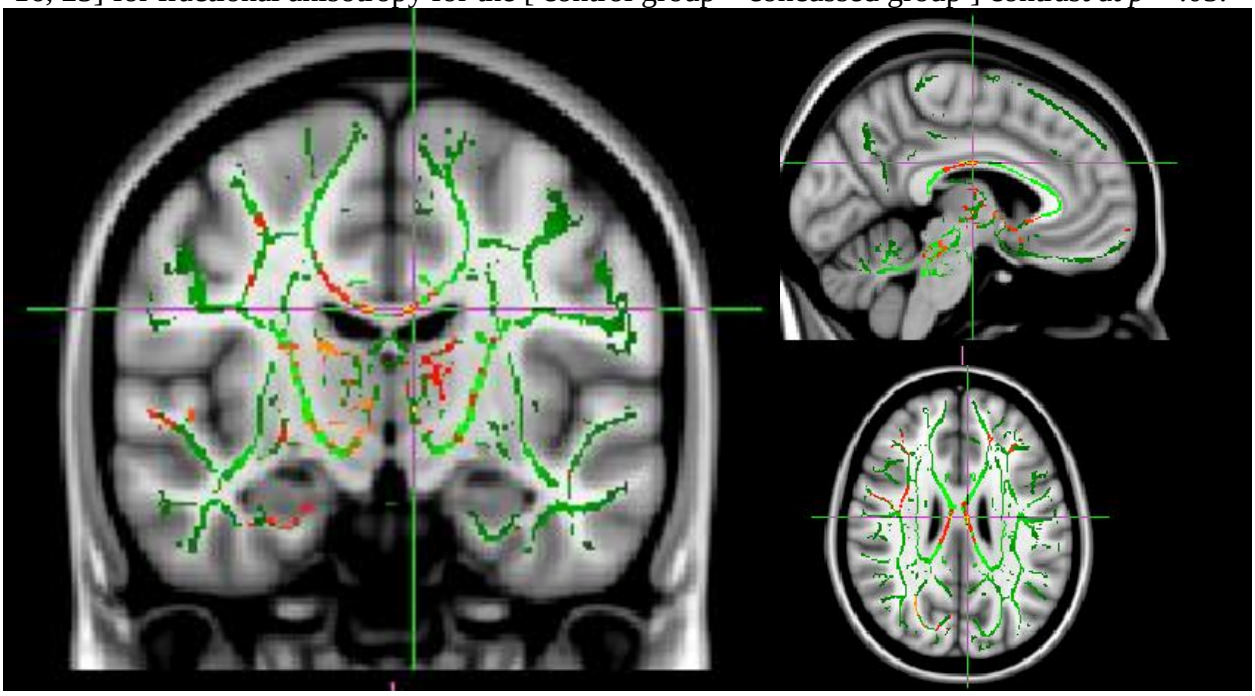
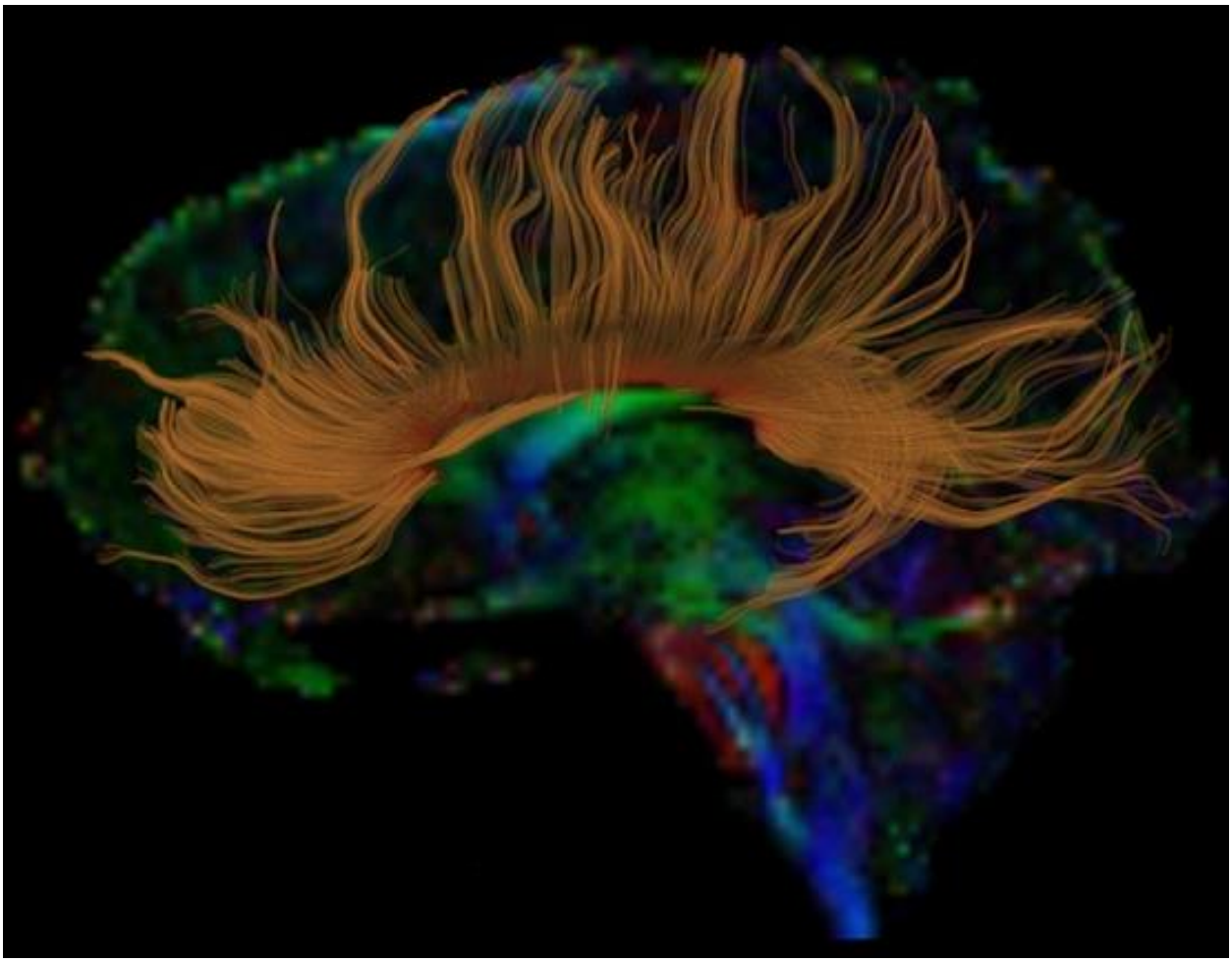


Figure 4.2 Fiber tractography of the corpus callosum for one participant overlaid on a color-by-orientation image from a diffusion tensor imaging scan.



CHAPTER 5

DISCUSSION

General Discussion

The current experiments examined neuroanatomical structures in middle-aged males who sustained at least two concussions in the context of their high school football experiences. Previous research has found structural and functional abnormalities in former professional athletes many years after they retired from their sport (De Beaumont et al., 2009; Strain et al., 2013; Tremblay et al., 2013). Such changes may be clinically meaningful, as repetitive head trauma may be associated with negative long-term outcomes, such as worse cognitive functioning, behavioral problems, and higher rates of neurodegenerative diseases (Guskiewicz et al., 2005; Gustkewicz et al., 2007; McKee et al., 2009). However, at the onset of this study, if was unclear if these structural abnormalities would also be evident in former football players who played in high school but did not play in college or professionally. The first study examined whether there are structural brain changes evident on traditional magnetic resonance imaging (MRI) scans between a group of former athletes who previously sustained multiple concussions compared to a group of former athletes that denied any form of concussive histories throughout their lifetime. The second study sought to elucidate potential white matter differences between the two groups using two diffusion tensor imaging (DTI) methodologies. Further, we examined how these neuroanatomical changes may be differentially associated with cognitive functioning between the groups. The specific findings have been discussed previously within their respective manuscripts. The main themes within the specific findings are presented below.

MRI approaches

From a biological perspective, we would expect to see volumetric changes associated with concussive injuries. The mechanism of a concussion is such that axons are stretched, twisted, and sheared due to the acceleration and deceleration forces exerted into the head upon impact. Animal models have shown that such a biomechanical injury triggers biochemical cascades of ions and neurotransmitters that try to restore balance in the cell, but ultimately lead to Wallerian degeneration and cell death (Giza and Hovda, 2001; Blennow, Hardy, and Zutterberg, 2012).

Many neuroimaging studies have reported positive results in their ability to find volumetric differences in those both acutely and chronically after concussive injuries (e.g., Gale et al., 1995; MacKenzie et al., 2002; Ross et al., 2012; Ross et al., 2014; Zhou et al., 2013; Jarrett et al., 2016; Cohen et al., 2007; Monti, et al., 2013), while few have published negative results (Ling et al., 2013). It is worth noting that many of the studies with positive volumetric findings have methodological differences to the current study. For instance, some of these studies recruited participants from an emergency department setting (e.g., Zhou et al., 2013), creating a sampling bias related to the severity of the concussion. Other studies required loss of consciousness with the concussion, while other studies recruited those with persistent concussive symptoms (e.g., Ross et al., 2012). Some studies analyzed more severe traumatic brain injuries along with those who received mTBIs (e.g., MacKenzie et al., 2002; Ross et al. 2014). Hofman et al. (2001) reported diffuse brain atrophy in participants with complicated concussions (i.e., positive neuroimaging findings at the time of the injury) after 6 months of recovery, but there was no atrophy noted in the scans of participants with non-complicated concussions.

These types of sampling biases in previously published studies may be driving their positive results and help to explain why the current study did not find such results. Given that the severity of brain injury has been linearly associated to the amount of volume loss seen on MRI scans (Ghosh et al., 2009, Levine et al., 2008), it may be that the previous studies, as a whole, have positive findings due to increased severity of injury. The only studies to show negative findings (i.e., Ling et al., 2013; Hofman et al. 2001) also included positive findings for others groups or analyses within the same study. These findings may exemplify the publication bias that has been well documented in the neuroimaging literature (Ioannidis, Munafò, Fusar-Poli, Nosek, & David, 2014; Jennings & Van Horn, 2012). One such study examined brain volumes in region on interest (ROI) analyses in psychiatric conditions (Ioannidis, 2011). After analyzing 41 meta-analyses, Ioannidis concluded that the number of studies with statistically significant results was statistically improbable, likely due to selective outcome reporting. This reporting bias also been shown when examining voxel-based morphometry studies across 324 individual studies in 47 meta-analyses (Fusar-Poli, et al., 2014). Interestingly, the less number of co-authors on the study, the larger the brain differences were between groups. Thus, it may be that there is no effect in the current study, which is discrepant with prior studies due to the sampling biases toward more severe concussions in previous studies combined with the scientific fields' bias toward publishing positive results while not publishing negative results (i.e., "file drawer problem"). The current study had a larger sample size compared to many previous studies, so we do not believe that the failure to find an effect is due an issue of statistical power. This is also supported by effect size analyses, which do not suggest group differences (i.e., $d = 0.01$ to $d = 0.17$).

This study may have also failed to find differences related to the control group comparison employed. Many prior control groups used control groups that were not exposed to

any form of head trauma. For instance, many studies with athletes used non-contact sport athletes or non-athletes as their control group, while studies that conducted in emergency departments used orthopedic injury controls. In the current study, the control group consisted of former football players who denied ever having receiving a frank concussion. This contrast isolates the unique effects of a definitive concussion. However, these control participants were subject to the repetitive brain trauma associated with playing a contact sport. Therefore, it is possible that both groups had similar brain changes that are associated with repetitive head trauma, which led to the two groups having similar brain volumes. There is a growing body of research that has shown brain changes with such repetitive head trauma in the absence of a concussive event (Koerte et al., 2012; Bazarian, 2014; Davenport et al., 2014).

DTI approaches

From an etiological perspective, changes in white matter microstructure are the direct mechanism of injury in concussive injuries (Giza and Hovda, 2001; Blennow, Hardy, and Zutterberg, 2012). Therefore, it is not surprising that most DTI studies show differences in white matter integrity in concussed individuals acutely after an injury (e.g., Arfanakis et al., 2002; Inglese et al., 2005; Miles et al. 2008; Lipton et al. 2009; Kumar et al. 2009). Further, a growing body of literature suggests that concussive injuries are associated with chronic white matter differences (e.g., Lipton et al., 2008; Niogi et al., 2008a; Niogi, et al., 2008b; Wada, Asano, and Shinoda, 2013)

The results of a whole-brain TBSS analysis in the current study show a diffuse array of clusters with reduced fractional anisotropy (FA). This type of method is data-driven in how it decides to spatially cluster the findings, and thus, is void of a specific anatomical hypothesis. As shown in Table 4.3, the regions that have lower FA values in the concussed group span several

white matter tracts across both hemispheres of the brain. Though these changes appear to be widespread, their clinical and functioning meaning is still unclear. Fractional anisotropy is the least specific of the DTI metrics in helping to explain potential underlying neuropathology. It is a proxy for overall white matter integrity, but can be reduced for a variety of reasons, such as cell death, edema, gliosis, changes in intracellular/extracellular fluid, changes in myelination, and crossing fibers (O'Donnell & Pasternak, 2015). In the absence of specific findings when examining other diffusion indices as well as the absence of cognitive differences between groups, it is unclear how to interpret this group difference.

Given that a whole-brain analysis is not driven by specific neuroanatomical hypotheses, we used tractography to analyze regions of interest (ROIs) based on implicated brain areas from previous literature. The three regions analyzed were the corpus callosum, the anterior limb of the internal capsule, and the uncinate fasciculus. Tractography analyses yielded higher radial diffusivity (RD) in the corpus callosum. Such higher RD values have been seen in animal studies with disrupted myelin formation (Song et al., 2002, 2003, 2005), which is consistent with the way RD is calculated based on the two vectors orthogonal to the predominant direction of diffusion (i.e., $(\lambda 2 + \lambda 3)/2$).

The corpus callosum (CC) has been implicated to be subject to white matter changes post-brain injury in various other studies (Arfanakis et al., 2002; Kumar et al., 2009; Zhang, et al., 2010). The CC is a topographically organized white matter tract that connects the left and right hemispheres. Its anterior portions (i.e., the genu) connect the prefrontal cortices, its middle parts connect the premotor, motor, and parietal cortices, and the posterior portion (i.e., the splenium) projects to the temporal and occipital cortices. A previous meta-analysis of DTI findings in concussive injuries reported lower FA and higher mean diffusivity (MD) in the

corpus callosum, and that this effect was driven by the posterior portions of the structure (Aoki et al., 2012). This is consistent with brain scans of more severe traumatic brain injuries that show significant damage to the splenium (Shiramizu et al., 2008; Colcombe, et al., 2011). The CC is likely vulnerable to such injuries due to its high organization in predominantly one direction. Lateral and rotational acceleration forces can greatly impact this structure due to the shear-strain on the axons, which preferentially affect in the prefrontal cortex, temporal pole, and occipital areas of the brain (Gentry, Godersky, & Thompson, 1988). Although some concussion literature implicated posterior regions of the CC, other studies have suggested anterior regions are more affected. For instance, Stamm et al. (2015) showed reduced FA in the anterior half of the tract, as well as increased RD in genu of the CC in retired professional football players who started tackling before the age of twelve as compared to a matched group that started tackling at age twelve and over. Future analyses of our data may divide the corpus callosum into anatomically-based segments to determine if there is a specific portion of the corpus callosum that differs between groups in this sample.

This study highlights how methodologies are important to consider in DTI research. For instance, there were group differences in the uncinate fasciculus and internal capsule using whole-brain analyses but not when using tractography. This finding is elaborated on in Chapter 4, but such a discrepancy exemplifies how one needs to be aware of these issues and let their theoretical questions drive the approach. We chose to use both methods in this study in order to both visualize white matter differences across the entire brain in a data-driven manner, as well as to test specific neuroanatomical hypotheses using tractography by examining the entirety of *a priori* defined white matter tracts.

Although we anticipated cognitive differences between groups and relationships between white matter indices and cognitive variables, there were no significant relationships between these variables. This is in contrast to several previous studies that found brain-behavior correlations in previously concussed samples (e.g., Miles et al., 2008; Lipton et al., 2009; Bazarian et al., 2007; Niogi, 2008b) such that worse white matter integrity (usually lower FA) was associated with worse overall cognition, memory, attention, or executive functioning. Given that our measures, methods, and sample size are comparable to those that found these effects, it may be that this sample is unique in that there are no measurable functional deficits despite neuroanatomical differences between the groups.

Conclusions

The current study demonstrates support that there are structural brain differences in individuals who sustained multiple concussions in the context of high school football. These differences were evident when examining white matter integrity, but did not appear to affect the size of the brain, specific structures, or neuropsychological functioning. These data support the notion that there may be subtle differences in the brains of individuals with multiple concussions, but these differences are not associated with functional deficits in cognitive ability. Future studies should continue to follow concussed high school players longitudinally, as well as assess athletes prospectively examining the brain health and cognitive functioning trajectories of athletes with and without concussions. Continued research on this topic will help inform clinical decision-making in young individuals who have sustained multiple concussions that may be deciding whether or not to keep playing contact sports.

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