THE IMPACT OF MULTIPLE CONCUSSIONS ON VERBAL MEMORY IN EX-ATHLETES: AN FMRI STUDY

by

DOUGLAS PRYSE TERRY

(Under the Direction of L. Stephen Miller)

ABSTRACT

This purpose of this study was to compare the neural activation during verbal memory processing between former athletes with a history of multiple concussions and former athletes without a concussive history using functional Magnetic Resonance Imaging (fMRI). It was hypothesized that those with concussive histories would have greater activation in brain regions related to language and memory during the task. However, results indicate hypoactivation in the concussed group compared to the non-concussed group in regions typically associated with verbal processing during both encoding and memory recall. These differences could not be accounted for by general memory ability or performance on the verbal memory fMRI task. Results suggest that multiple concussions sustained earlier in life may be associated with subtle underlying changes in the verbal memory encoding system that limits one from accessing higher-order semantic networks. These differences may represent biomarkers for late-life functional decline.

INDEX WORDS: concussion, mild traumatic brain injury, memory, fmri, functional imaging
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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Reports from the Centers for Disease Control and Prevention indicate approximately 207,000 concussions, or mild traumatic brain injuries (mTBIs), occur in America each year (CDCP, 2007). This is likely an underestimate as it is based on medically diagnosed concussions where loss of consciousness (LOC) was exhibited. However, studies report that LOC is only exhibited in about 10% of sports related mTBIs (Guskiewicz, Weaver, Padua, & Garrett, 2000; Macciocchi, Barth, Alves, Rimel, & Jane, 1996). Other estimates suggest 1.6 to 3.8 million sports related concussions occur each year (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).

Concussions are known to cause a variety of well-documented symptoms, including headaches, dizziness, and fatigue (McCrory et al., 2009), irritability, anxiety, and impaired neuropsychological functions such as reduced attention, concentration, and memory problems (Arciniegas et al., 2000; Hall, Hall, & Chapman, 2005). These symptoms can resolve within hours, days, or months (McCrory et al., 2009; Guskiewicz et al., 2003; Schretlen & Shapiro, 2003; Frencham, Fox, & Mayberry, 2005), but may be persistent at 1-year follow-up (Jacobson, 1995; Dikmen, McLean, & Temkin, 1986; Rutherford, Merrett, & McDonald, 1978). Posttraumatic migraines (i.e. a combination of headaches, nausea, and sensitivity to light or noise) have been associated with poorer cognitive functioning and prolonged recovery time (Kontos, et al., 2013). Literature suggests between 8% (Binder, 1986) and 33% (Rimel et al.,
1981) of people with history of mTBI suffer from long-term physical, psychological, and cognitive difficulties, commonly known as Post-Concussive Syndrome (PCS), which inhibits people from functioning at pre-morbid levels. Cognitive symptoms of PCS include attentional and executive functioning deficits, psychomotor slowing, and reduced verbal and working memory. Frequently cited somatic symptoms consist of headaches and fatigue (Ahman, Saveman, Styrke, Bjornstig, Stalnacke, 2013). Depressed mood, anxiety, irritability, agitation, poor motivation, social withdrawal, interpersonal difficulties, and other psychiatric symptoms are also frequently reported (Al Sayegh, Sandford, & Carson, 2010; Binder, 1986). Many people who have sustained a TBI also exhibit deficits in executive functioning, which is the ability to adapt to changing situations and appropriately regulate behavior (Tate, 1999). However, the existence of PCS as a unique disorder has become a controversial topic in the literature as many symptoms of PCS overlap with other psychiatric and neurological disorders. For example, 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).

One prospective study that examined people with mTBI found a 50% to 100% increase over base rates in affective disturbance at three-years post-injury (Fann et al., 2004). However, the absence of visible brain damage makes these patients a therapeutic challenge for their health-care providers. It also raises the suspicion of purposeful symptom exaggeration and malingering for those patients that may be involved in litigation over their injury (Green, Iverson, & Allen, 1999).

While still controversial, a newer body of studies suggests the possibility of long-term neuropsychological deficits in people with concussions. Many studies have reported that athletes recover from concussive symptoms and on neuropsychological batteries within two weeks of
injury (Macciocchi et al., 1996; McCrea et al., 2003). However, retrospective studies suggest sequela of an mTBI can be measured after the acute recovery period. Many of these studies looking at the chronic neuropsychological effects of concussive injuries have been plagued with confounding factors, thus limiting one’s ability to know if deficits are caused by the injury itself. For example, the use of alcohol when sustaining the head injury may affect long-term outcomes (Kraus & Nourjah, 1988). People who show symptoms of PCS and experience cognitive difficulties are likely to have greater neurological insult (Blumbergs et al., 1994; Dikmen, Machamer, & Temkin, 2001) or have psychological or litigation factors that may contribute to their prolonged symptoms (Binder, 1986; Dikmen & Levin, 1993).

**Neuropsychological Findings**

Thus far, the findings of long-term outcome studies are mixed. At an average of 6-years post-injury, no subjective impairment was reported from university students who had mild concussive histories (Segalowitz, Bernstein, & Lawson, 2001); however, the concussion group performed more slowly and less accurately on oddball tasks and exhibited reduced P300 amplitudes, which suggests subtle attention and information processing may persist long after the original injury. In another study, group-matched controls scored better than people with self-reported concussion at an average of 8-years post-injury on a Digit-Symbol substitution task and on a more difficult dual task involving tone discrimination and visual working memory (Bernstein, 2002). Additionally, the concussed group exhibited smaller P300 amplitudes on both an easy and a difficult auditory discrimination task, suggesting that the combination of electrophysiological and neuropsychological might reveal subtle long-term abnormalities associated with an mTBI.
In another study, Vanderploeg and colleagues examined the neuropsychological outcomes of people with concussions at an average of eight years after their injury (Vanderploeg, Curtiss, & Belanger, 2005). These participants were community-dwelling male veterans who were non-referred and did not have pending legal proceedings. They were compared to demographically-matched normal controls and motor vehicle accident controls who had never sustained a head injury. Although no group differences on a standard neuropsychological test battery of 15 measures were found, the mTBI group showed subtle problems with attention as evidenced by lower rates of continuation to completion on the Paced Auditory Serial Addition Test (PASAT) and in excessive proactive interference (PI) on the California Verbal Learning Test (CVLT). Unique to the mTBI group, PASAT continuation problems were associated with left-sided visual imperceptions, and excessive PI was associated with impaired tandem gait. These results suggest that concussion may have adverse long-term neuropsychological outcomes on subtle aspects of complex attention and working memory. A follow-up study on the same sample examined prevalence of long-term psychiatric, neurologic, and psychosocial morbidities when controlling for demographic characteristics, comorbid medical conditions, and early-life psychiatric problems (Vanderploeg, Curtiss, Luis, & Salazar 2007). Compared with uninjured controls, those with mTBI histories had higher rates of depression and PCS, as well as peripheral visual imperceptions and impaired tandem gait. The concussion group also had poorer psychosocial outcomes, including an increased likelihood of self-reported disability, lower income, and marital problems.

Konrad et al., (2010) found medium to large effect sizes in the cognitive domains of learning, delayed memory, working memory, attention, and executive functioning at 6-years post-concussion due to MVA. In this study, 33 individuals who acutely sought professional
medical attention after sustaining an mTBI were assessed at an average of six years post injury. Participants were matched to healthy controls and administered a comprehensive neuropsychological battery consisting of several tests that spanned five cognitive domains. The mTBI group did worse across all domains, specifically on working memory tasks (effect size $d = 0.98$), executive functioning ($d = 0.8$) and episodic memory recall tasks ($d = 0.71$). Those who exhibited structural brain abnormalities or a negative response bias (as assessed by the Word Memory Test) were excluded. Effect sizes could not be accounted for by self-perceived deficits, depression, compensation claims, or negative response bias. Self-reported symptoms of depression scores were significantly higher in the patient group, and three patients fulfilled DSM-IV criteria for a mild episode of major depression.

However, other studies have not yielded these long-term cognitive differences in people with mTBI (Dikmen, Ross, Machamer, & Temkin, 1995). For example, Ettenhofer & Abeles (2009) reported that mTBI does not result in cognitive impairment or psychiatric dysfunction, evidenced by a lack of neuropsychological differences shown across several domains of functioning as determined by follow-up testing performed 2.9 years after injury. Several other previous studies also failed to find neuropsychological differences after the acute phase of recovery (e.g., Echemendia, Putukian, Macklin, Julian, & Shoss, 2001; Macciocchi, Barth, Wayne, Rimel, & Jane, 1996; for review see Carroll et al., 2004).

As previously mentioned, many studies examining neuropsychological outcomes of mTBI after the acute phase of recovery were plagued with methodological issues such as differences in diagnostic criteria for a concussion, possible exaggeration or malingering due to pending litigation, the use of purely symptomatic samples, and a lack of attention toward preexisting and comorbid psychosocial factors (Rohling et al., 2011). For instance, one study that
examined the long-term cognitive effects of a single concussion at least one year post-injury found that those with an mTBI without a PCS diagnosis functioned just as well as two control groups (one with PCS-like symptoms and one without symptoms; Dean & Sterr, 2013). However, participants who sustained an mTBI with a PCS diagnosis had worse processing speed and working memory compared to the other groups. Authors have turned to meta-analyses to accurately assess long-term outcomes in this population.

Several meta-analytic studies have been conducted on this topic, all of which found negligible effects sizes of a single concussion after a minimum of 3 months of recovery (Binder, Rohling, & Larrabee, 1997, \(d=-0.07\); Schretlen & Shapiro, 2003, \(d=0.04\); Frencham, Fox, & Mayberry, 2005, \(d=-0.11\); Belanger & Vanderploeg, 2005, \(d=-0.04\)). Recently, the individual studies included in Binder, Rohling, & Larrabee (1997) and Frencham & Shapiro (2005) were re-analyzed to determine whether a subgroup of mTBI patients had a significantly poorer outcome than the majority of the sample (Pertab, James, & Bigler, 2009). The authors intended to assess separately the method of injury, the diagnostic criteria employed, the type of neuropsychological assessment tool employed, and whether the sample was symptomatic or non-symptomatic. Unfortunately, they only had sufficient data to the type of neuropsychological tool employed. Like prior meta-analytic researchers, there was a non-significant effect size after 90 days of recovery when collapsing the various neuropsychological domains and tests. However, when examining each neuropsychological test separately, they found that the concussed group scored lower than the control group, exhibiting effect sizes of 0.81 for Verbal Paired Memory tests, 0.35 for Coding tasks, and 0.31 for Digit span tasks. These analyses show that although some neuropsychological domains appear to return to premorbid levels during the post-acute stage of recovery, there may be some differences that can be measured reliably in the learning, memory,
and processing speed domains. This meta-analysis also speaks to the heterogeneity of the literature and the need for more research on this topic.

The most recent meta-analysis on this literature employed a random effects model to examine 25 of the most empirically sound studies. Authors concluded that overall neuropsychological functioning of those with a history of a single concussion is comparable to controls at 3 months post-injury when collapsing across neuropsychological domains (Rohling et al., 2011). However, when each cognitive domain was examined separately, Working Memory remained impaired after 3 months of recovery with a modest effect size of 0.19 (Rohling et al. 2011).

Literature also 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). Common in retired boxers and
other former athletes, CTE typically manifests several years 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). 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).

There is still much debate over whether or not there are long-term, lingering effects associated with two or more concussions. History of at least two concussions has been associated with poorer performance on tests measuring executive functioning and processing speed (Collins et al., 1999), 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). 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; Macciochi, Barth, Littlefield, & Cantu, 2001). Retired football players with a history of three or more concussions are shown to be five times more likely to have received a diagnosis of mild cognitive impairments (MCI) and three times more likely to
self-report significant memory impairments in comparison to retirees without a history of concussion (Guskiewicz et al., 2005).

Neuropsychological studies have examined the long lasting effects of multiple concussions in this manner. One study examined former college athletes between the ages of 50 and 65 (mean 60.79) who sustained their last concussion in early adulthood (mean age = 26.05; De Beaumont et al., 2009). These individuals had a lifetime history of between one and five mTBIs. Participants completed the Rey Complex Figure Test (RCFT), where they copied a figure, and were asked to draw the figure from memory three minutes (immediate memory) and 30 minutes (delayed memory) after the initial copy. Participants were also asked to perform a modified arrow version of the computerized Eriksen flanker task, where one responds to the direction of a target arrow while ignoring distracter arrows that point either in the same or the opposite direction. Relative to controls, former athletes with concussive histories had lower performance on neuropsychological tests of visual episodic memory and selective attention/executive functioning/response inhibition, providing evidence for chronicity of cognitive changes related to concussion.

Neuropsychological impairment appears to be common across several sports where concussions are present. For instance, professional soccer players exhibited lower scores in memory, planning, and visuoperceptual processing when compared to professional athletes who do not engage in contact sports (Matser, Kessels, Jordan, Lezak, & Troost; 1998). Additionally, performance on these tasks was inversely related to the number of concussions sustained while “heading” the ball within the soccer group. Rugby players with three or more previous concussions have shown long-term processing speed deficits on traditional and computerized
neuropsychological measures as compared to their non-concussed colleagues (Gardner, Shores, & Batchelor, 2010).

Studies have compared the neuropsychological performance of people with multiple concussions to those that have only received one concussion to try and elucidate the cumulative effects of concussions. A recent meta-analysis evaluated data from ten studies, which examined a total of 614 multiple mTBI cases and 926 one mTBI control cases using varied neuropsychological tests from multiple cognitive domains (Belanger, Spiegel, & Vanderploeg, 2010). Each of these 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 (\(d = .16\)). This finding was used as the basis for a portion of the current experiment’s design. Preliminary work from our laboratory shows only subtle effects of post-acute multiple concussions in young adults (Terry et al., 2012). These subtle group differences are only apparent at liberal statistical thresholds.

**Functional Imaging Findings**

Due to these potential lingering and long-term effects, mTBIs have been studied using functional neuroimaging to examine the pathophysiological and functional sequelae associated with these injuries. Functional imaging techniques, such as single photon emission computed tomography (SPECT), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have recently become popular in neurorehabilitation research as they permit the
exploration of the dynamic changes that take place in the brain in response to cognitive tasks.

fMRI is a specifically powerful tool because it is non-invasive, repeatable, has relatively high spatial resolution, and has a temporal resolution that allows one to examine transient cognitive events (McAllister, Sparling Flashman, & Saykin, 2001), which is especially helpful for tracking symptom permanence and resolution following a head injury. Furthermore, fMRI may be better able to reveal the subtle changes in brain functioning than structural scans alone (Ptito, Chen, Johnston, 2007).

Functional MRI operates on the basis that changes in blood flow and blood oxygenation in the brain are closely linked to neural activity, as active neuronal cells increase their consumption of oxygen. Utilizing the need to replenish oxygen to activated regions of the brain, blood oxygenation level dependent (BOLD) responses allow one to track cerebral blood flow by relying on the difference in magnetic properties between oxyhemoglobin and deoxyhemoglobin. Oxygenated hemoglobin is diamagnetic, as it has zero magnetic moment and does not cause disturbances in magnetic fields, whereas deoxygenated hemoglobin is paramagnetic, and is attracted to the magnetic field and causes magnetic field disturbances. Given that neural activity requires the utilization of oxygen, when brain regions become engaged, oxygenated hemoglobin is depleted and quickly replenished to the active neural area. This increase in the ratio of oxygenated to deoxygenated hemoglobin decreases the paramagnetic effects of the deoxygenated hemoglobin, resulting in a greater BOLD signal. Researchers have theorized that increased BOLD activation indicates that the brain has to work harder to complete the same task (McAllister et al., 1999). Moreover, a greater dispersion of activation could imply the necessitation of recruitment of outside areas to complete tasks. Both possibilities suggest that BOLD contrast has a potential for uncovering altered brain functioning in human subjects.
Due to these potential lingering and long-term effects of concussive injuries, single mTBIs have been examined with fMRI to elucidate differences in the allocation of neural resources. Previous neuroimaging studies examining working memory in healthy participants have shown greater cognitive load is associated with increased dorsolateral prefrontal cortex (DLPFC) activity in normal populations (Manoach et al., 1997). Concussed individuals within 1-month of injury exhibited similar working memory performances to control participants, but hyperactivation in the DLPFC and in right parietal regions during the moderate processing load condition (2-back; McAllister et al., 1999). A follow-up study utilized a high processing load condition (3-back), where the concussed group showed less of an increase in activation compared to the control group, even though behavioral results were still comparable (McAllister et al., 2001). The curvilinear relationship suggested here has also been reported in persons with schizophrenia, where increases in activation are shown in moderate load conditions with a comparative absence of increased activation in high load conditions (Fletcher et al., 1998). This suggests that those with acute mTBI may allocate most of their neural resources in the moderate working memory load condition and therefore have minimal activation increases during higher cognitive load tasks. Another n-back paradigm on concussed athletes within 1 week of injury demonstrated hyperactivation at the time of their fMRI scan was associated with a more prolonged clinical recovery than athletes who did not demonstrate hyperactivation (Lovell et al., 2007). A longitudinal fMRI study showed n-back hyperactivation at three time points during concussion recovery (3 days, 2 weeks, and 2 months; Dettwiler et al., 2014). These differences suggest that patients with history of mTBI require more neural resources to perform a given task, though behaviorally they perform at levels comparable to controls. The idea that compensatory mechanisms utilize alternative cognitive resources has previously been supported by imaging
studies of more severe brain injuries. (Levine et al., 2002; Ricker, Hilary, & DeLuca, 2001; Mani, Miller, Yanasak, & Macciocchi, 2007).

Additionally, increased BOLD signal change in the hippocampus and DLPFC has been shown post-mTBI using fMRI during working memory tasks, in addition to an increase in activity outside of the regions of interest (Zhang et al., 2010). The mTBI group had additional activation in the left DLPFC during the encoding phase of spatial navigation working memory task that was not observed in normal controls. Given that greater activation is believed to represent increased functional effort, thus making it a potential marker for brain dysfunction, there is much promise for research aimed at identifying abnormal cerebral functioning and the point at which these abnormalities become permanent. In a prospective study where college football players with a recent mTBI completed an fMRI finger-sequencing task pre- and post-injury, increases in the amplitude and extent of activation were observed in the supplementary motor cortex, premotor cortex, parietal cortex, and cerebellum despite lack of deficits in behavioral measures, suggesting an increased amount of neural resources were necessary to appropriately complete the task (Jantzen, Anderson, Steinberg, & Scott-Kelso, 2004).

Despite evidence suggesting hyperactivation in neural networks following a concussion, some studies have showed task-related hypoactivation compared to control participants. In a version of an externally ordered working memory task, athletes who received two through five concussions and were at least 1 month post-injury with active PCS had decreased BOLD signal changes within the right DLPFC, an area shown to be involved in the monitoring of information during working memory processes (Chen et al., 2004). Anecdotally, one participant in this study with PCS symptoms was scanned several times, demonstrating that as symptoms improved, activation patterns resembled that of the control group. A follow-up study showed that at an
average of 5 months after mTBI, participants with continued post-concussive symptoms responded more slowly to working memory tasks. Additionally, the mTBI group had less fMRI brain activation in the hypothesized regions of interest than the control group (Chen et al., 2007). However, the mTBI group’s activation extended into posterior regions of the brain, including the left temporal lobe. This supports the idea that compensatory mechanisms may be playing a role in this process. Kontos et al. (in press) showed that college athletes who sustained a concussion three weeks prior to their assessment showed hypoactivation in multiple brain regions across a battery of functional tasks. Further, a study examining working memory in concussed teenagers 9-90 days post-injury showed hypoactivation in the DLPFC, anterior cingulate, premotor cortex, supplementary motor cortex, and other regions (Keightley, et al., 2014).

Resting state fMRI in asymptomatic athletes at 10 days post-injury showed subtle suggested reduced inter-hemispheric connectivity in the primary visual cortex, hippocampal, and DLPFC networks (Slobounov et al., 2011) and a spatial memory task performed by asymptomatic concussed participants within 30 days of injury showed significantly larger activation patterns in the left parietal cortex and right DLPFC (Slobounov et al., 2010) Additional regions of activation in the left DLPFC and cerebellum were activated during memory encoding that was not observed in the matched controls.

To our knowledge, only five functional imaging studies have examined the post-acute neural effects of mTBI. Two of these studies examined purely symptomatic populations. Despite similar behavioral data across groups, an externally ordered working memory paradigm showed attenuated activation in a symptomatic concussed group at 6-months post-injury in the putamen, the body of the caudate nucleus, the right thalamus, and in the DLPFC bilaterally, where more severe symptoms had lower BOLD signal changes in the right mid-DLPFC (Gosselin et al.,
Similarly, a group of symptomatic mTBI participants at 2-years post-injury showed reduced regional cerebral blood flow bilaterally in the thalamus using true fast imaging with steady state precession arterial spin labeling, which was significantly correlated with neurocognitive dysfunction in several cognitive domains (Ge et al., 2009). However, in a sample that included both symptomatic and asymptomatic college athletes, there were minimal neuropsychological differences after at least 6-months of recovery (mean = 18 months) as well as no statistically significant differences in neural activation across a motor, a response inhibition, and a working memory paradigm (Terry et al., 2012). This suggests relative plasticity of younger adults cognitive ability following multiple concussions.

Two recent studies examined the impact of concussions several years after they occurred. Middle-aged individuals with a history of one or more medically diagnosed concussions before the age of 25 showed worse memory performance, smaller bilateral hippocampi, and reduced neural activity during memory performance in cortical regions important for memory retrieval such as the middle PFC, precentral gyrus, and other regions (Monti, et al., 2013). In an fMRI study examining episodic memory in retired NFL athletes, players who reported three or more concussions exhibited hyperactivation in the PFC compared to players with zero, one, or two concussions. However, the “high” concussion group showed hypoactivation in memory related regions such as the right parahippocampal gyrus and bilateral inferior parietal cortex (BA 40) compared to the control group (Ford, Giovanello, & Guskiewicz, 2013).

**Present Study**

The present study examined verbal memory in individuals who have sustained at least two concussions, but have not received a concussion in at least fifteen years, in hopes to better understand the long-term effects of multiple concussive head injuries sustained in early sports
related activities. This experiment employed functional magnetic resonance imaging (fMRI) in order to define changes in brain activation associated with a history of multiple concussive injuries. Neuropsychological tasks were used to gauge the relationship between neurocognitive ability and fMRI defined brain activation. This is the first study to our knowledge to examine the chronic effects of concussions in former high school football players using functional MRI methodologies.

The concussion group was compared to a matched control group who has never received a concussion. Authors expect that participants who suffered from multiple concussions will have lower neuropsychological performances on tasks that relate to delayed memory but have similar behavioral scores on the fMRI paradigm when compared to the control group. Additionally, participants with a history of multiple concussions are hypothesized to recruit more neural resources in an attempt to appropriately complete the tasks. Therefore, Aim #1 of this study was to compare activation across the entire brain and in *a priori* regions of interest commonly associated with verbal memory tasks. Specifically, it was thought that the concussion group would exhibit a greater magnitude of activation and greater spatial extent of brain activation within these *a priori* regions when compared to their non-concussed counterparts. Additionally, Aim #2 of this study was to examine compensatory mechanisms that may uniquely be associated with the mTBI groups. We hypothesized that additional areas of activation outside *a priori* regions would be present in the concussion group that was not evident in control participants.
CHAPTER 2

METHOD

Participants

Power Analysis

A previous fMRI study conducted simulations to effectively determine how many subjects and how many image samples are needed to obtain an acceptable statistical power when accounting for the millions of voxels in the brain. The purpose of this study was to obtain estimates of percent signal change and the two sources of variability from fMRI data, and then use these parameter estimates in simulation experiments in order to generate power curves (Desmond & Glover, 2002). Intra-subject variability was estimated from resting conditions, and inter-subject variability and percent signal change were estimated from verbal memory data. When estimating a significant signal change as 0.75% or greater, twenty-five subjects are needed to achieve 80% power at an alpha of 0.000002, which is a realistic alpha value after controlling for multiple comparisons across the brain. As the current study is examining verbal working memory, this study is appropriate to reference. However, as it only used one group, their findings were extrapolated to the current study that plans to contrast two separate groups. We planned to collect data on a sample of 20 participants in each group (i.e., mTBI and controls) for a total of 40 participants, as this many participants has been shown to detect meaningful differences between clinical samples using a similar verbal memory paradigm (Bookheimer et al., 2000; Braskie, Small, & Bookheimer, 2007).
Recruitment/Exclusionary Criteria

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 age 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 magnetic resonance imaging (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, had 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), current use of any psychotropic medications, bipolar disorder, or schizophrenia. One hundred and thirty nine potential participants from the surrounding Athens area community were contacted regarding participation. Of those, 41 participants were recruited and consented. However, imaging data is only available for 36 participants (see Table 2.1). Upon completion, participants were given $50 for their participation.

Participants were divided into two groups: one with a history of two or greater concussions and whose most previous concussion was more than fifteen years prior to examination (n=25); and one without any concussive history (n=16). 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 is diagnosed when at least one of the following criteria is 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 consistence (\(\alpha=0.82\)) 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 with high one-month test-retest reliability (Kendler, Jacobson, Myers, & Eaves, 2007; Moffitt, et al., 2007). Groups were matched on age [t(39) = 1.01, \(p = 0.28\)], education [t(39) = 0.28, \(p = 0.78\)], and pre-morbid IQ level [t(39) = -0.63 , \(p = 0.53\)] based on independent samples t-test analyses (Table 3.1).

**Materials and Procedure**

This study is a portion of a larger investigation that is currently being conducted in the University of Georgia Neuropsychological and Memory Assessment Laboratory. As a result, the measures and experimental paradigm are within an extensive behavioral and neuroimaging protocol. This investigation only represents a portion of the measures and procedures that were 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, participants signed a University of Georgia Institutional Review Board approved informed consent document. Participants were told participation is voluntary and the study could be discontinued at any time.

Participants completed a concussion symptom checklist to index current, if any, post-concussive symptoms. The Symptom Assessment Scale (SAS) is a 22-item symptom list where the participant ranked 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 level of performance on each trial and consistency of 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 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 administered 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 was used to control for the potential confound of premorbid intellectual functioning differences between the concussed and control group.

Participants completed the California Verbal Learning Test – Second Edition (CVLT-II), a verbal learning and memory task that assesses both the recall and recognition of word lists over immediate and delayed memory trials. This involved the oral presentation of two 16-word lists; the first list contained four words from each of four semantic categories (vegetables, animals, furniture, and ways of traveling). Participants repeated as many words from the list that they could remember following each of the five list presentations. The second list, which contained different words from similar and different semantic categories (vegetables, animals, musical instruments, and parts of a house), was then read aloud one time. Immediate recall of the new list was assessed, followed by recall of the initial list. After 20 minutes, delayed recall of the repeated list and delayed recognition of both lists were also assessed. This measure has previously been administered in a variety of adult populations and has a scoring system that allows raw scores to be converted to a normal z-distribution based on age and gender where mean = 0.0 and standard deviation = 1.0 (Strauss, Sherman & Spreen, 2006). Split-half reliability for total trial scores (trail 1 + 3 vs. trails 2 + 4, etc.) was high for the normative sample \( r = 0.82 \) and for a mixed clinical sample \( r = 0.83 \) (Delis, Kramer, Kaplan, & Ober, 2000).
Split-half reliability examining the number of times each of the 16 words on the repeated list were recalled across the five immediate-recall trials was 0.79 for the normative sample and 0.83 for a clinical sample. The CVLT-II correlates well with its predecessor (CVLT; \( r = 0.76 \) for Total Recall of Trails 1-5; Delis, Kramer, Kaplan, & Ober, 2000). This measure allowed for group comparisons of verbal learning/memory functioning.

Participants also completed the Logical Memory I / II subtests from the Wechsler Memory Scales – 4th Edition (WMS-IV; Weschler, 2009). In this subtest, participants listened to two short story narratives read by the examiner and immediately repeated as many details from the story as possible. Participants also recalled the stories after a longer delay and answered forced-choice recognition questions that pertain to the stories. Logical Memory I and II were both shown to have high split-half reliability coefficients, thus demonstrating internal consistency (\( r = 0.82 \) and \( r = 0.85 \), respectively). These subtests are highly correlated with their predecessors from WMS-III (corrected \( r = 0.75 \) and 0.76 for Logical Memory I and II, respectively). The logical memory subtests moderately correlate with the CVLT-II scores (\( r = 0.38-0.51 \)), but are thought to measure different components of memory as determined by a factor analysis that was completed during WMS-IV development. It was concluded that the information gleaned from these two tests are not redundant and may measure different aspects of memory (Weschler, 2009). Participants in this study also completed several additional measures that were not examined in the current analysis.

**fMRI Experimental Design**

Participants also underwent a two-hour MRI scanning session at the BioImaging Research Center at the University of Georgia. While in the MRI scanner, subjects performed a learning task involving unrelated pairs of words that is particularly sensitive for the identification
of damage to the medial temporal lobe (Rausch & Babb, 1993) and shown to engage memory systems maximally, as adopted by Bookheimer et al. (2000) and Braskie et al (2007). In this test, subjects viewed pairs of words (e.g., UP and FOOT; TABLE and FLOWER) for ten separate periods, or encoding epochs, which lasted 19.5 s each. There were a total of 10 word-pairs in the paradigm, each of which was presented a total of five times. Each encoding epoch only contained five of the word-pairs. All words were presented in white on a black screen. Initially, the first word of the word-pair was presented alone (1 s) and then accompanied by the second word of the word-pair (2 s). Participants pressed a button on the response pad with their index finger when they saw the second word of the word-pair appear on screen to validate that they were attending to the stimuli. This also served to control for motor activity in other conditions. A black screen (0.9 s) followed each word-pair. The five word-pairs in each encoding epoch were pseudo-randomly selected in a pre-determined manner such that all of the words were presented one time during epochs 1+2, one time during epochs 3+4, etc. Therefore, each of the ten word-pairs was presented a total of five times. The word-pairs were presented in a fixed order across participants. The list contained six two-syllable words and fourteen one-syllable words, randomly combined into ten pairs. Thirteen of the twenty words in the list were nouns and seven were adjectives. No word in the list was a common associate of any other word in the list, based on word association norms (Nelson, McEvoy, & Schreiber, 2004).

Each encoding block was followed by a distracter task (19.5 s), where participants attended to a series of letters (e.g. “XXXXX – YYYYY”) to mimic the active stimulus. This was included to discourage rehearsal. Participants pressed with their right index finger when they saw these letters to control for motor activity in the other conditions. Then, during each 19.5 s recall epoch, participants saw the first word of each pair (e.g. UP - _____; TABLE - _____) and
attempted to recall the second word silently (3 s) and then viewed a black screen (0.9 s). Subjects responded using pre-specified finger response to indicate perceived success or failure at recalling the second word. After each recall epoch, participants focused on a crosshair (6 s) to allow for brain activity to return to its baseline. There were 10 encoding, 10 response, and 10 baseline epochs. Before each new epoch began, there was a 3 s prompt to inform the participant which task they would need to perform. The full presentation of the task lasted 12 m and 24 s. Total correct number of perceived successful recalls for each subject were summed across the ten recall epochs, where participants achieved scores between 0 and 50. Additionally, immediately after scanning, participants were asked to freely recall as many words as they could from the paradigm. They received credit each time they verbalized a “second word” from the word-pair (Post-Task free recall, maximum score=10). They were also prompted to say the second word from each word-pair after the experimenter orally read the first word of the pair (Post-Task cued recall, maximum score=10).

**MRI Acquisition**

A 3.0T General Electric (GE) Signa HDx magnetic resonance system was used to collect anatomical and functional images. 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.

Blood oxygenation level dependent (BOLD) signals were collected during the verbal memory task. Stimuli were presented using E-Prime software (version 1.2, Psychology Software Tools, Inc., Pittsburgh, PA), and delivered via MRI compatible goggles (Resonance Technology Inc.) at a resolution of 800 x 600 pixels. Participants responded using MRI compatible response
pads (Cedrus LUMINA); reaction times and responses were recorded by E-Prime during all functional runs.

Functional scans were acquired axially along the AC-PC line using a T2*-weighted single shot echo planar imaging (EPI) sequence (4 mm slice thickness; 30 interleaved axial slices; TR = 1500 ms; TE = 25 ms; 90° RF pulse; matrix size = 64x64; FOV = 220 x 220 mm; in-plane resolution, 220 x 64mm). Four dummy sample images were recorded and discarded at the beginning of each run to allow longitudinal magnetization to reach equilibrium. A high-resolution 3D T1-weighted fast spoiled gradient recalled echo (FSPGR) anatomical scan was also acquired to be used in the post-processing of BOLD fMRI images (TR = 7.5 ms; TE = min full; FOV = 256 x 256 mm matrix; flip angle = 20°; slice thickness = 1.2 mm; 154 axial slices); acquisition lasted 6 m 20 s. Additionally, several other scan sequences were collected on participants, which are not included in the current analysis. Total scanning time was approximately 55 minutes.

**Functional MRI Data Reduction and Analysis**

Data were processed and analyzed using Statistical Parametric Mapping (SPM12, Wellcome Department of Cognitive Neurology, London, UK). Images were first converted from GE DICOM format to NIFTI using the dcm2nii conversion tool (Rorden, 2007). Each subject's data was slice time corrected to account for the interleaved acquisition. Data then underwent realignment and unwarping procedures to adjust for any distortion that may have resulted from magnetic field inhomogeneities and movement during the scan. The anatomical scan was co-registered to the functional images through a transformation process, and then was segmented into grey matter, white matter, and cerebrospinal fluid, which aided the normalization of functional images into normal space. Functional images were normalized to the Montreal
Neurological Institute (MNI) template using a nonlinear, 12-parameter affine transformation registration, and smoothed with a 6.75-mm FWHM Gaussian filter to de-emphasize random noise and increase the signal-to-noise ratio. Following pre-processing, 1st level analyses identified the conditions and timing of the paradigm. A Matlab file (i.e., .mat) was used to identify the onset of the blocks in seconds.

For the verbal memory task, activation maps using the General Linear Model (SPM12) included encoding vs. baseline contrasts and retrieval vs. baseline contrasts. Contrasts were then subjected to 2nd level, random effect analysis on the group level. Another set of random effects analyses compared these contrasts between groups. Activation of control participants and mTBI participants were subtracted from each other using several two-sample t-tests to identify differential activation at the statistical threshold uncorrected $p < .005$ with a minimum of 20 contiguous voxels, which has been shown to be equivalent to a False Discovery Rate (FDR) of $p < .05$ and achieve a more desirable balance between Type 1 and Type 2 error rates in fMRI data than more conservative approaches (Lieberman & Cunningham, 2009). Similar thresholds have been used in recent literature examining memory and/or concussive injuries (Ford et al., 2013; Jacques, Rubin, & Cabeza, 2012). Specifically, the contrasts that were examined: 1) Concussion encoding – Control encoding, 2) Control encoding – Concussion encoding, 3) Concussion retrieval – Control retrieval, 4) Control retrieval – concussion retrieval).

Region of Interest (ROI) analyses (WFUPickAtlas; Maldjian, Laurienti, Burdette, & Kraft 2003; Maldjian, Laurienti, & Burdette, 2004) were performed for the verbal memory task using the cluster level interference method (statistical threshold $p < 0.005$, 10 contiguous voxels). ROIs were identified a priori based on previous literature (Dobbins & Wagner, 2005; Wagner et al. 1998; Mitchell, Johnson, Raye, & D’Esposito, 2000; Mitchell, Johnson, Raye, Mather, &
D’Esposito, 2000), and included 1) hippocampus/parahippocampal gyri, 2) middle temporal gyrus, 3) inferior temporal gyrus, 4) fusiform gyrus, 5) DLPFC, and 6) VMPFC.

**Behavioral data analysis**

To analyze accuracy during the verbal memory task, a between-groups t-test was conducted using SPSS version 16.0 (SPSS, Chicago) to identify group differences in total accuracy scores (range: 0-50) from data obtained during the fMRI scanning session as well as post-task free recall (range: 0-10) and post-task cued recall (range 0-10). Neuropsychological data were compared across groups to assess differences in cognitive functioning. A correction of 5 comparisons was made for the number between group individual analyses of separate neuropsychological measures.
Figure 2.1 Flow chart of recruitment

- **Contacted (n=139)**
  - Not interested or excluded (n=98)
    - Not interested (n=44)
    - Out of age range (n=12)
    - Played college football (n=11)
    - Only one concussion (n=9)
    - MRI Incompatible (n=8)
    - Left handed (n=8)
    - Concussions outside of football (n=6)
  - Could not scan (n=5)
    - Claustrophobia (n=4)
    - MRI incompatible (n=1)
  - Scanned (n=36)
  - Enrolled (n=41)
    - Played college football (n=11)
    - Only one concussion (n=9)
    - MRI Incompatible (n=8)
    - Left handed (n=8)
    - Concussions outside of football (n=6)
CHAPTER 3

FMRI HYPOACTIVATION DURING VERBAL LEARNING AND MEMORY IN EX-ATHLETES WITH MULTIPLE CONCUSSIONS

\(^1\) Terry, D. P., Adams, E., Ferrara, M. S., & Miller, L. S. To be submitted to the *Archives of Clinical Neuropsychology.*
Abstract

Research suggests that a history of multiple mild traumatic brain injuries (mTBIs) early in life may be associated with late life memory deficits. This study examined the neural activation associated with verbal encoding and memory retrieval processes in former athletes ages 40-65 who received at least two concussions (mean=4.3) while playing high school football as identified by structured interview. The experimental group was compared to non-concussed past players matched on age, education, and estimated premorbid IQ. Functional magnetic resonance images (fMRI) were collected during a modified verbal paired associates paradigm. Results indicated that those with concussive histories had hypoactivation in left hemispheric regions associated with language processing, including the inferior/middle frontal gyri, angular gyrus, lateral occipital lobe, planum temporale (Wernicke’s area), supramarginal gyrus, and orbitofrontal cortex, compared to controls. However, concussive history was not associated with worse memory functioning on neuropsychological tests or worse behavioral performance during the fMRI paradigm. These results suggest that multiple concussions sustained earlier in life may be associated with subtle underlying changes in the verbal memory encoding system that limits one from accessing higher-order semantic networks, but that this difference does not translate into measurable cognitive performance deficit.
**Introduction and Literature Review**

Reports from the Centers for Disease Control and Prevention indicate approximately 207,000 concussions, or mild traumatic brain injuries (mTBIs), occur in America each year (CDCP, 2007). This is likely an underestimate since concussions are often not diagnosed unless loss of consciousness (LOC) is exhibited, which only occurs in 10-20% of sports-related mTBIs (Guskiewicz, Weaver, Padua, & Garrett, 2000; Macciocchi, Barth, Alves, Rimel, & Jane, 1996). Other estimates suggest 1.6 to 3.8 million sports related concussions occur each year (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 U.S. (Thurman, 2001).

Concussions are known to cause a variety of symptoms, including headaches, dizziness, and fatigue (McCrory et al., 2009), irritability, anxiety, and cognitive difficulties such as reduced attention, concentration, and memory problems (Arciniegas et al., 2000; Hall, Hall, & Chapman, 2005). These symptoms most often resolve within hours, days, or months (McCrory et al., 2009; Guskiewicz et al., 2003; Schretlen & Shapiro, 2003; Frencham, Fox, & Mayberry, 2005), but, in some cases, may be persistent at 1-year follow-up (Jacobson, 1995; Dikmen, McLean, & Temkin, 1986; Rutherford, Merrett, & McDonald, 1978).

A newer body of studies suggests the possibility of long-term cognitive deficits in people with concussions. Some studies indicate generally intact functioning on traditional neuropsychological assessments, but subtle attentional and working memory difficulties several years post injury (Segalowitz, Bernstein, & Lawson, 2001; Bernstein, 2002; Vanderploeg, Curtiss, & Belanger, 2005; Vanderploeg, Curtiss, Luis, & Salazar 2007). These subtle deficits are often accompanied by worse psychosocial outcomes and higher rates of mood symptomatology. Other studies have found more severe differences. For example, at six year
post-injury, people with a history of concussion did significantly worse across all domains of cognition when compared to non-concussed participants (working memory $d = 0.98$, executive functioning $d = 0.8$, episodic memory recall tasks ($d = 0.71$; Konrad et al., 2010). However, other studies have not yielded these long-term cognitive differences in people with mTBI (Dikmen, Ross, Machamer, & Temkin, 1995; Ettenhofer & Abeles, 2009; Echemendia, Putukian, Macklin, Julian, & Shoss, 2001; Macciocchi, Barth, Wayne, Rimel, & Jane, 1996; for review see Carroll et al., 2004). Several meta-analytic studies found negligible effects sizes of a single concussion after a minimum of 3 months of recovery (Binder, Rohling, & Larrabee, 1997, $d = 0.07$; Schretlen & Shapiro, 2003, $d = 0.04$; Frencham, Fox, & Mayberry, 2005, $d = 0.11$; Belanger & Vanderploeg, 2005, $d = 0.04$). The most recent and methodologically rigorous meta-analysis found a statistically significant but subtle deficit associated with working memory tasks ($d = 0.19$ Rohling et al., 2011). However, when examining each neuropsychological test separately, it was also indicated that previously concussed individuals scored lower than the control participants (e.g. $d = 0.81$ for Verbal Paired Memory tests; Pertab, James, & Bigler, 2009).

Given that those with a history of mTBI are at a greater risk for having a second concussion (Guskiewicz et al., 2003), examination of the cognitive effects of multiple concussions has also been an area of research. 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). 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).

Due to these potential long-term effects, mTBIs have been studied using functional MRI (fMRI) to examine the pathophysiological and functional sequelae associated with these injuries. FMRI is a specifically powerful tool because it is non-invasive, repeatable, has relatively high spatial resolution, has a temporal resolution that allows one to examine transient cognitive events (McAllister, Sparling Flashman, & Saykin, 2001) and may be better able to reveal the subtle changes in brain functioning than structural scans alone (Ptito, Chen, & Johnston, 2007).

Concussed individuals within 1-month of injury exhibited hyperactivation in the dorsolateral prefrontal cortex (DLPFC) and in right parietal regions during a moderately difficult working memory task despite comparable behavioral performance (2-back; McAllister et al., 1999). During a more difficult condition (i.e. 3-back), the concussed group showed less of an increase in activation (McAllister et al., 2001), suggesting that those with acute mTBIs may allocate most of their neural resources in the moderately difficult condition and therefore have minimal activation increases during higher cognitive load tasks. Other n-back paradigms demonstrated that hyperactivation in concussed participants at the time of their fMRI scan was associated with a
longer clinical recovery than athletes who did not demonstrate hyperactivation (Lovell et al., 2007). This activation pattern was shown to persist longitudinally for at least two months (Dettwiler et al., 2014). Hyperactivation was also evident during motor programming and spatial memory fMRI tasks (Jantzen, Anderson, Steinberg, & Scott-Kelso, 2004; Slobounov et al., 2010). An increased extent of activation not seen in control subjects has also been shown in concussed participants, such as in the hippocampus and DLPFC during a working memory task (Zhang et al., 2010). These differences suggest that patients with an acute mTBI require more neural resources to perform a given task despite comparable behavioral performance.

However, some studies show task-related hypoactivation compared to control participants. Kontos et al. (in press) revealed that college athletes showed hypoactivation in multiple brain regions across a battery of functional tasks at three weeks post-injury. Paradigms assessing working memory have also shown hypoactivation compared to controls (Keightley, et al., 2014; Chen et al., 2004; Chen et al., 2007). However, one of these studies suggested compensatory mechanisms due to an increased extent of activation outside typical task-related regions (Chen et al., 2007).

Studies assessing the long-term brain activation differences of mTBI have yielded mixed findings. A symptomatic concussed group at 6-months post-injury showed hypoactivation in several regions during a working memory task (Gosselin et al., 2011). Similarly, a group of symptomatic mTBI participants 2-years post-injury showed reduced regional cerebral blood flow bilaterally in the thalamus using arterial spin labeling, which was significantly correlated with neurocognitive dysfunction in several domains (Ge et al., 2009). However, in a sample that included symptomatic and asymptomatic college athletes, there were no statistically significant fMRI activation differences across motor, response inhibition, and working memory paradigms.
(Terry, et al., 2012). In a recent paper, middle-aged individuals with a history of one or more concussions before the age of 25 had worse memory performance, smaller hippocampi, and reduced neural activity during memory performance in cortical regions important for memory retrieval (Monti, et al., 2013). Further, former NFL players who reported three or more concussions exhibited hyperactivation in the PFC compared to players with zero, one, or two concussions (Ford, Giovanello, & Guskiewicz, 2013). However, the “high” concussion group showed hypoactivation in memory related regions such as the parahippocampal gyrus and inferior parietal cortex.

The present study examined verbal memory in individuals who sustained at least two football-related concussions, but have not received a concussion in at least fifteen years, in hopes to better understand the long-term effects of multiple head injuries. This is the first study to our knowledge to examine the chronic effects of multiple concussions in former high school football players using functional MRI methodologies. Authors expected participants who suffered from multiple concussions to have lower neuropsychological performances on tasks related to delayed memory, but have similar behavioral scores on the fMRI paradigm when compared to the control group. Additionally, participants with a history of multiple concussions were hypothesized to recruit more neural resources in an attempt to appropriately complete the tasks.

**Method**

**Participants**

Former high school football players were recruited from a suburban southeastern community though newspaper advertisements, online advertisements, and news articles about the study, or were contacted by researchers using emails and phone calls based on information gained though public records and football alumni listservs. Participants were included if they
were right-handed, male, and age 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 magnetic resonance imaging (MRI) environment, younger than 40 years, or older than 65 years. They were also excluded if they reported being illiterate, left-handed, learning English as a second language, a history of alcohol or drug abuse/dependency within the past five years, a history of significant 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, bipolar disorder, or schizophrenia. Participants were provided with a small monetary compensation for their time.

Forty-one participants were ultimately enrolled in the study. However, imaging data is only available for 36 participants due to claustrophobia during the task (n=4) and late identified MRI incompatibility (n=1). Participants were divided into two groups: one with a history of two or greater concussions and whose most recent concussion was more than fifteen years prior to examination (n=25); and one without any concussive history (n=16). Concussions were identified using a two-step processes that included a self-report questionnaire followed by an in-depth, empirically-based semi-structured interview. A self-report questionnaire was developed to identify concussive history based on criteria set by the American Congress of Rehabilitation Medicine (Medicine, 1993), where a mTBI is diagnosed when at least one of the following criteria is met after a 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 then 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 shown to have moderate to high internal consistence ($\alpha=0.82$) 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 with high one-month test-retest reliability (Kendler, Jacobson, Myers, & Eaves, 2007; Moffitt, et al., 2007). People with exactly one concussion were not included in the study. Groups were matched on age [$t(39) = 1.01, p = 0.28$], education [$t(39) = 0.28, p = 0.78$], and pre-morbid IQ level [$t(39) = -0.63, p = 0.53$] based on independent samples $t$-test analyses (Table 3.1).

**Procedures**

After determining eligibility via a phone screen, participants were scheduled for either one or two research sessions depending on their personal preference. The commitment totaled four to five hours and encompassed informed consent, concussion interview, self-report measures, neuropsychological testing, MRI safety screening, and MRI scanning. Before engaging in the fMRI task, participants learned how to complete the task using a computer outside of the MRI environment. Participants were in the MRI scanner for approximately 55 minutes, during which structural, functional, and phase/magnitude images were collected among other scan sequences not relevant for this study.
Measures

Symptom Assessment Scale (SAS). A 22-item concussion symptom checklist to index current, if any, post-concussive symptoms. The participant ranked 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 (MSVT). This short, computerized verbal memory test was used to quantify each participant’s memory and symptom validity based on level of performance on each trial and consistency of responding over trials to assess for adequate task engagement. (Green, 2004). Participants must have achieved a score considered to show effortful participation (85%) to continue the study. All participants (n=41) met or exceeded this threshold on the four main indices (immediate recognition, delayed recognition, consistency, and paired associates).

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). Participants pronounced words of increasing difficulty. This was used to control for the potential confound of premorbid intellectual functioning differences between the concussed and control group. Raw scores are converted to standard scores that account for age, gender, race, education, and word reading performance.

California Verbal Learning Test – Second Edition (CVLT-II) was used to assess verbal learning and memory over immediate and delayed memory trials. Participants listened to word lists read orally and then repeated as many words from the list as they can remember. Recall of
the initial list of words occurred following each of the five list presentations, after hearing a new list of words, and after a 20-minute delay. Recognition of the list is also tested. Split-half reliability for total trial scores (trail 1 + 3 vs. trails 2 + 4, etc.) was high for the normative sample ($r = 0.82$) and for a mixed clinical sample ($r = 0.83$) (Delis, Kramer, Kaplan, & Ober, 2000).

Raw scores are converted to normed scores that account for age and gender.

*Logical Memory I / II, Wechsler Memory Scales – 4th Edition (WMS-IV).* To assess contextual memory, participants listened to two short story narratives and immediately repeated as many details from the story as possible (Weschler, 2009). Participants were also asked to recall the stories after a longer delay and to answer forced-choice recognition questions that pertain to the stories. Logical Memory I and II were both shown to have high split-half reliability coefficients, thus demonstrating internal consistency ($r = 0.82$ and $r = 0.85$, respectively). Raw scores are converted to normed scores that account for age and gender. Participants in this study also completed several additional measures that will not be examined in the current analysis.

**Neuroimaging**

*Task.* While in the MRI scanner, subjects performed a learning task involving unrelated pairs of words that is particularly sensitive for the identification of damage to the medial temporal lobe (Rausch & Babb, 1993) and shown to engage memory systems maximally, as adopted by Bookheimer et al. (2000) and Braskie, Small, & Bookheimer (2007). In this task, subjects viewed pairs of words (e.g., UP and FOOT) for ten separate periods, or encoding epochs, which lasted 19.5 s each. There were a total of 10 word-pairs in the paradigm. Each encoding epoch contained five of the word-pairs. All words were presented in white on a black screen. Initially, the first word of the word-pair was presented alone (1 s) and then accompanied by the second word of the word-pair (2 s). Participants pressed a button on the response pad with
their index finger when they saw the second word of the word-pair appear on screen to validate that they were attending to the stimuli (Figure 3.1). This also served to control for motor activity in other conditions. A black screen (0.9 s) followed each word-pair. The five word-pairs in each encoding epoch were pseudo-randomly selected in a pre-determined manner such that all of the words were presented one time during epochs 1+2, one time during epochs 3+4, etc. Therefore, each of the ten word-pairs was presented a total of five times. The word-pairs were presented in a fixed order across participants. The list contained six two-syllable words and fourteen one-syllable words, randomly combined into ten pairs. Thirteen of the twenty words in the list were nouns and seven were adjectives. No word in the list was a common associate of any other word in the list, based on word association norms (Nelson, McEvoy, & Schreiber, 2004).

Each encoding block was followed by a distracter task (19.5 s), where participants attended to a series of letters (e.g. “XXXXX – YYYYY”) to mimic the active stimulus. This was included to discourage rehearsal. Participants pressed with their right index finger when they saw the letters to control for motor activity in the other conditions. Then, during each 19.5 s recall epoch, participants saw the first word of each pair (e.g. UP - _____) and attempted to recall the second word silently (3 s) and then viewed a black screen (0.9 s). Subjects responded using pre-specified finger responses to indicate perceived success or failure at recalling the second word. After each recall epoch, participants focused on a crosshair (6 s) to allow for brain activity to return to its baseline. There were 10 encoding, 10 response, and 10 baseline epochs (Figure 3.2). Before each new epoch began, there was a 3 s prompt to inform the participant which task they would need to perform. The full presentation of the task lasted 12 m and 24 s. Total correct number of perceived successful recalls for each subject was summed across the ten recall epochs, where participants achieved scores between 0 and 50. Additionally, immediately
after scanning, participants were asked to freely recall as many words as they could from the paradigm. They received credit each time they verbalized a “second word” from the word-pair (Post-Task free recall, maximum score=10). They were also prompted to say the second word from each word-pair after the experimenter orally read the first word of the pair (Post-Task cued recall, maximum score=10).

**MRI Acquisition.** A 3.0T General Electric (GE) Signa HDx magnetic resonance system was used to collect anatomical and functional images. This scanner was 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. Functional scans were acquired axially along the AC-PC line using a T2*-weighted single shot echo planar imaging (EPI) sequence (4 mm slice thickness; 30 interleaved axial slices; TR = 1500 ms; TE = 25 ms; 90° RF pulse; matrix size = 64x64; FOV = 220 x 220 mm; in-plane resolution, 220 x 64mm). Four dummy sample images were recorded and discarded at the beginning of each run to allow longitudinal magnetization to reach equilibrium. A high-resolution 3D T1-weighted fast spoiled gradient recalled echo (FSPGR) anatomical scan was also acquired to be used in the post-processing of BOLD fMRI images (TR = 7.5 ms; TE = min full; FOV = 256 x 256 mm matrix; flip angle = 20°; slice thickness = 1.2 mm; 154 axial slices); acquisition lasted 6 m 20 s.

**Functional MRI Data Reduction and Analysis.** Data were processed and analyzed using Statistical Parametric Mapping (SPM12b, Wellcome Department of Cognitive Neurology, London, UK). Images were converted from GE DICOM format to NIFTI using the dcm2nii conversion tool (Rorden, 2007). Each subject's data was slice time corrected to account for the interleaved acquisition. Data then underwent realignment and unwarping procedures to adjust for any distortion that may have resulted from magnetic field inhomogeneities and movement during
The anatomical scan was co-registered to the functional images through a transformation process, and then was segmented into grey matter, white matter, and cerebrospinal fluid, which aided the normalization of functional images into normal space. Functional images were normalized to the Montreal Neurological Institute (MNI) template using a nonlinear, 12-parameter affine transformation registration, and smoothed with a 6.75-mm x 6.75mm x 8mm FWHM Gaussian filter to de-emphasize random noise and increase the signal-to-noise ratio.

For the verbal memory task, activation maps using the General Linear Model (SPM12b) including the following contrasts for each subject: encoding vs. baseline; retrieval vs. baseline. Contrasts were subjected to 2nd level, random effect analysis on the group level. Another set of random effects analyses compared these contrasts between groups. Whole-brain activation maps of control subjects and mTBI subjects were subtracted from each other using two-sample t-tests to identify differential activation at the statistical threshold of $p < .005$, uncorrected with a minimum of 20 contiguous voxels, as this has been shown to be equivalent to a False Discovery Rate (FDR) of $p < .05$ and achieve a more desirable balance between Type 1 and Type 2 error rates in fMRI data than more conservative approaches (Lieberman & Cunningham, 2009). Similar thresholds have been used in recent literature examining memory and concussive injuries (Ford et al., 2013; Jacques, Rubin, & Cabeza, 2012).

Region of Interest (ROI) analyses (WFUPickAtlas; Maldjian, Laurienti, Burdette, & Kraft 2003; Maldjian, Laurienti, & Burdette, 2004) were performed for the verbal memory task using the cluster level interference method (statistical threshold $p < 0.005$, 10 contiguous voxels). ROIs were identified a priori based on previous literature (Dobbins & Wagner, 2005; Wagner et al. 1998; Mitchell, Johnson, Raye, & D’Esposito, 2000; Mitchell, Johnson, Raye, Mather, &
D’Esposito, 2000), and included 1) hippocampus/ parahippocampal gyri, 2) middle temporal gyrus, 3) inferior temporal gyrus, 4) fusiform gyrus, 5) DLPFC, and 6) VMPFC.

**Behavioral data analysis.**

To analyze accuracy during the verbal memory task, a between-groups t-test was conducted using SPSS version 16.0 (SPSS, Chicago) to identify group differences in total accuracy scores (range: 0-50) from data obtained during the fMRI scanning session as well as post-task free recall (range:0-10) and post-task cued recall (range 0-10). Neuropsychological data were compared across groups to assess differences in cognitive functioning. A correction of 5 comparisons was made for the number between group individual analyses of separate neuropsychological measures.

**Results**

**Concussion symptomatology and neuropsychological measures**

Those in the concussion group reported an average of 4.3 concussions [SD = 3.7, range = 0-15; median = 3 concussions]. When two extreme values in number of reported concussions were reduced to the value of two standard deviations above the mean, the average number of concussions was 3.9. LOC was accompanied with 29% of the concussions, while 43% of the concussions were associated with either anterograde or retrograde amnesia (Table 3.1). Medical attention was reportedly sought following 29% of the concussions. Both groups endorsed several current symptoms associated with concussion [control group M = 3.4, SD = 3.0; concussion group M = 4.8, SD = 4.3]. T-test analyses failed to find group differences between the total number of current symptoms endorsed [t(39) = -1.18, p = .24], the average duration of these symptoms [t(39) = -0.19, p = .85], and the average severity of these symptoms [t(39) = -0.87, p = .39] (Table 3.1).
Independent samples t-tests failed to reveal group differences on several neuropsychological measures (Table 3.2). The concussion group was not statistically different from the control group when learning a list of words across repeated trials [CVLT-II Trials 1-5 T-score: \( t(39) = 0.37, p = .72 \)], recalling the list of words after a short delay [CVLT-II Short Delay Z-score: \( t(39) = 0.12, p = .91 \)], and recalling the list of words after a 20-minute delay [CVLT-II Long Delay Z-score: \( t(39) = 0.38, p = .71 \)]. Similarly, recall of two stories was comparable between groups both immediately after the story was read and after a 20-minute delay [WMS-IV LM I scaled score: \( t(39) = -0.13, p = .90 \); WSM-IV LM II scaled score: \( t(39) = -0.23, p = .83 \)]. Significance levels did not change when the five participants who did not undergo MRI scanning were excluded from the analyses.

**FMRI Task**

Bivariate correlation between self-reported successful recall during the behavioral paradigm and objective memory recognition of the word pairs after the paradigm showed a strong association \([r = .52, p < .001]\), suggesting that the participants’ responses in the scanner were accurate in estimating memory performance. Independent t-test analyses showed no differences in accuracy between groups for subjective memory during the paradigm \([t(34) = 0.54, p = .59]\), for post-task free recall \([t(34) = 1.23, p = .23]\), or for post-task cued recalled \([t(34) = 0.97, p = .34]\) (Table 3.3; Figure 3.3).

**Functional Imaging**

For the Encoding-view contrast, within group analyses of concussion group yielded widespread activation at the threshold of \( p < .05 \), Family-Wise Error (FWE) corrected and a minimum of eight contiguous voxels. Of the eleven significant clusters, the largest area of activation was in the left inferior frontal gyrus, pars opercularis (BA44), which spread into the
precentral gyrus [Peak T = 11.56]. The left paracingulate and left fusiform corticies also showed activation. Other areas of activation included the bilateral cerebellum and the left visual cortex (V1, V3, and V4) that extended into the left lateral occipital cortex and occipital pole (Table 3.4).

In the Encoding-view contrast, the control group had significant fifteen clusters at the threshold of \( p < .05 \), FWE corrected, greater than eight contiguous voxels. The largest peak activation cluster was in the left superior parietal lobe [peak T = 17.29]. Other areas of activation included the left middle/inferior frontal gyrus (pars opercularis and triangularis), left fusiform gyrus, left fusiform, left paracingulate gyrus, left premotor, left inferior temporal gyrus, and the bilateral cerebellum.

At the threshold \( p < .005, 20 \) contiguous voxels, the concussion group had no regions of hyperactivation compared to the control group (Table 3.5). However, the control group exhibited more activation than the concussion group in five clusters, including the left middle frontal gyrus/inferior frontal gyrus, the left angular gyrus, the left lateral occipital lobe, planum temporale (Wernicke’s area), the left supramarginal gyrus, and the left orbitofrontal cortex. Exploratory post-hoc analyses of the \textit{a priori} ROIs revealed hyperactivation in the control group compared to the concussion group during the encoding task in the left orbitofrontal cortex (maxima at MNI -50, 26, 6; k=49, Peak T=3.32) and the left middle temporal gyrus (maxima at MNI -50, -64, 12; k=34, Peak T=3.10) at uncorrected \( p < .005, 20 \) contiguous voxels. There were no areas of hyperactivation in the concussed group over the control group in the ROI analyses.

The Recall-view condition also revealed diffuse activation. The concussed group showed activation in twelve clusters, the largest in magnitude being in the bilateral paracingulate gyrus [Peak T = 12.15] at a threshold of FWE corrected \( p < .05, 8 \) contiguous voxels (Table 3.6). Other
areas of activation include the bilateral orbitofrontal cortex, left inferior frontal gyrus (BA44/BA45), left middle temporal gyrus, left precentral gyrus, left lateral occipital cortex, the right cuneus/V2, left precuneus, right thalamus, and the bilateral cerebellum.

Widespread activation was similarly seen in the control group during the Recall-view condition, which also yielded twelve clusters of significant activation at a threshold of FWE corrected $p < .05$, 8 contiguous voxels. Areas of activation included the bilateral paracingulate gyrus, the bilateral insula, left inferior gyrus, bilateral middle frontal gyri, the right insula, the left angular gyrus, the left lateral occipital cortex, the right thalamus, and the right cerebellum.

At the threshold of $p < .005$, 20 contiguous voxels, the concussion group did not show any areas of increased activation compared to the control group (Table 3.7). However, the control group showed more activation during Recall-view in the left middle frontal gyrus compared to the concussed group. Exploratory post-hoc ROI analyses failed to show any activation related to the recall contrast.

**Discussion**

The purpose of this study was to examine BOLD activation in middle-aged former high school football players who reported a history of multiple concussions compared to non-concussed players in response to a verbal memory paradigm. In addition, this study compared aspects of verbal memory functioning in these individuals as measured by standard neuropsychological tests given the literature’s previous discrepant findings. Overall, we found similar patterns of activation between the two groups, with notable differences in neural recruitment evident. These data suggest that sustaining multiple mTBIs as an adolescent may be associated with modest long-term alterations in brain activity during memory encoding and retrieval.
These subtle differences are meaningful given the nature of the two participant groups. The experimental group was matched to the control group on age, education, and estimated premorbid IQ. Further, differences in verbal learning and recall were not evident on traditional neuropsychological measures, nor were there differences in the behavioral performance on the task during the functional imaging. Given that both perceived and objective accuracy on the memory paradigm were equivalent between the concussed and non-concussed participants, the ability to attribute potential differences in brain activation during the encoding and retrieval processes were not confounded by differences in behavioral performance.

Overall, participants in both the concussed and non-concussed groups recruited similar brain regions during both the encoding and the retrieval task. After subtracting out the distractor (i.e. “View”) condition from the encoding blocks, both groups showed activation in left frontal areas such as the inferior frontal gyrus, temporal areas like the fusiform cortex, and subcortical areas such as the paracingulate gyrus. Further, both groups showed sensory related activation in the occipital lobe likely due to the increased salience and visual processing of the active stimuli as compared to the distractor task. Both groups also showed activation in the cerebellum, which has previously been implicated in working memory tasks and various other cognitive functions (Marvel & Desmond, 2010).

However, the between-group comparison for the encoding task revealed the non-concussed group showed greater activation in several left hemisphere regions, including the middle frontal gyrus, inferior frontal gyrus, the orbitofrontal cortex, the planum temporale (Wernicke’s area), the angular gyrus, and the supramarginal gyrus. These regions have previously been implicated in the “levels-of-processing” model of language functioning (Craik & Lockhard, 1972), which suggests that encoding more complex information requires additional
cortical resources and therefore increases retention. The encoding of more complex verbal information has previously been associated with increased BOLD signal in the left inferior frontal, left prefrontal, left inferior parietal, middle temporal, and right supramarginal regions (Bonner-Jackson, Csernansky & Barch, 2007; Henson, Hornberger, & Rugg, 2005). Our results suggest that concussed individuals may have subtle underlying changes in their verbal memory encoding system that limits them from accessing higher-order semantic networks. For instance, the control group may have used verbally mediated encoding strategies to a greater extent than the concussed group.

The recall condition also showed similar activation patterns for both groups. Consistent areas of activation included the paracingulate gyrus, the left inferior frontal gyrus, the right orbitofrontal cortex, the left precentral gyrus, the right thalamus, the left lateral occipital cortex and the bilateral cerebellum. However, the concussion group showed hypoactivation compared to the control group in the left middle frontal cortex. Previous research suggests that the left middle frontal cortex is associated with memory recall, and becomes progressively more activated during the retrieval of perceptually detailed information that was previously encoded (Ranganath, Johnson, & D’Esposito, 2000). Given the complexity of the verbal paired associates task, this suggests that those with concussive histories may have alterations in both encoding and retrieval networks associated with higher order verbal processing. Further, this pattern of hypoactivation related to concussive history is consistent with other functional imaging studies that similarly examined the long-term effects of concussions several years after the injury (Ge et al., 2009; Ford et al., 2013; Monti et al., 2013). However, this is the first study to find these effects in a cohort of former athletes who did not play for a college or professional team.
Despite similar accuracy in behavioral performance on the verbal memory paradigm according to independent samples t-tests, effect size analyses may help explain the differences in neural activation. Calculating effect sizes shows that the control group outperformed the concussed group by approximately one-third of a standard deviation (Post-task Free Recall, Cohen’s $d = 0.36$; Post-task Cued Recall, Cohen’s $d = 0.32$). This difference was not statistically significant on the t-test analyses likely due to the small sample size, but the magnitude of effect size suggests that the concussed group may have a subtle memory and recall disturbance that helps to explain their pattern of hypoactivation in the fMRI paradigm compared to controls.

There is a strong association between worse behavioral performance and decreased brain activity has been shown in other patient populations, which could either be due to a dysfunction in the cortical network of interest (e.g. worse memory functioning) or other methodological factors (e.g. divergent cognitive processes, differential motivational effects, other confounding factors; Manoach, 2003).

The findings in this study do not appear to be affected by current concussion symptomatology. The SAS has previously discriminated between a group of athletes who had a history of multiple concussions and a group of nonconcussed athletes (Terry et al., 2012). However, given the relatively common nature of these symptoms (e.g. headache, fatigue, sleep disturbance), both groups endorsed experiencing several symptoms over the 24 hours prior to testing. Therefore, the hypoactivation exhibited by the concussed group is likely not an artifact of their symptomatology. Additionally, it calls to question the chronicity of post-concussive symptoms in individuals who experienced concussions prior to finishing high school.
**Limitations and Strengths**

There are some caveats to acknowledge in this study. History of concussions was measured in a retrospective way. However, these instances of potential head injuries occurred at least fifteen years ago. Thus, memory ability as well as nonspecific factors (e.g. personality, history since concussion, interest in football, knowledge of concussions) may have influenced participants’ self-report of previous concussions. Further, two participants in the concussion group (8% of the concussion group) reported a history of more than ten concussions. Several of these concussions were confirmed by an examiner using the ACE, thus validating membership to the multiple concussion group. However, accuracy of all concussions was unable to be documented due to the participants’ difficulty recalling the events. These issues related to retrospective reporting plague many studies of this nature. Validating concussions by examining medical records may be useful, but many concussions would be excluded due to the underreporting of symptoms. For example, participants in this study reported only receiving professional medical attention after 29% of their concussions, which is consistent with a more modern estimate that 50% of all football-related concussion go unreported (McCrea, Hammeke, Olsen, Leo, & Guskiewicz, 2004). Additionally, these nonspecific personality factors may also have influenced study participation given that this sample was collected by convenience. Those who have increased salience related to their high school football experience or head injuries may have self-selected to be a part of the study.

This study was also cross-sectional. Although the previously concussed group and the non-concussed controls were matched on several variables thought to influence our findings, it is possible that the groups differed on factors other than their history of head injuries. Longitudinal studies would more accurately show the chronic effects of mTBIs as a product of aging. Like
other studies in this field, the sample size was small due to the specialized nature of the participants. This can lead to a reduced ability to detect true differences on some conditions. Additionally, this highly specialized sample (i.e. middle aged men who received two or more football related concussions before graduating high school) limits the generalizability of these findings. Future studies need to replicate the current findings and help clarify the nature of concussions in other samples across other age groups.

Despite these limitations, this study had many strengths. For instance, unlike several previous studies, this study used an empirically based assessment tool to confirm the presence of two or more concussions and to screen out concussive histories in the control group. This study also employed a measure of task engagement, on which all participants performed adequately. This suggests the participants were putting forth sufficient effort on the remainder of testing and were not trying to exaggerate or malinger symptoms as previous studies have warned (Green et al., 1999).

**Conclusions**

The current study is the first to our knowledge to examine the long-term consequences of sports-related concussions in former high school athletes using functional imaging. Results on formal memory tests suggest that players with a history of multiple concussions perform just as well as a group of matched, non-concussed teammates. Further, history of concussions did not appear to have an effect on the behavioral performance on a verbal memory task during functional imaging. However, the concussion group had a modestly different pattern of neural activity associated with both verbal encoding and memory retrieval such that they showed several clusters of hypoactivation in brain regions traditionally associated with language and memory functioning. Such differences suggest that multiple concussions sustained early in life
may lead to differential recruitment of neural regions. Future research is needed to examine the longitudinal pattern of these differences as well as how they may affect players later in older adulthood, particularly as to how this may or may not influence later functional ability.
References


Echemendia, R. J., Putukian, M., Mackin, R. S., Julian, L., & Shoss, N. (2001). Neuropsychological test performance prior to and following sports-related mild traumatic


Table 3.1 Group Demographics

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=16)</th>
<th>Concussed (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>49.1 (8.3)</td>
<td>52.0 (8.05)</td>
<td>.28</td>
</tr>
<tr>
<td>Years of Education</td>
<td>15.0 (2.2)</td>
<td>15.2 (2.3)</td>
<td>.78</td>
</tr>
<tr>
<td>WTAR</td>
<td>108.3 (12.5)</td>
<td>110.6 (11.1)</td>
<td>.53</td>
</tr>
<tr>
<td>Number of concussions</td>
<td>-</td>
<td>4.3 (3.7)</td>
<td>.53</td>
</tr>
<tr>
<td>Concussions with LOC</td>
<td>-</td>
<td>29.0%</td>
<td></td>
</tr>
<tr>
<td>Concussions with medical attention</td>
<td>-</td>
<td>29.0%</td>
<td></td>
</tr>
<tr>
<td>Concussions with memory lapse</td>
<td>-</td>
<td>43.0%</td>
<td></td>
</tr>
<tr>
<td>SAS Number of symptoms</td>
<td>3.4 (3.0)</td>
<td>4.8 (4.3)</td>
<td>.24</td>
</tr>
<tr>
<td>SAS Average Duration</td>
<td>2.0 (1.7)</td>
<td>2.1 (1.4)</td>
<td>.85</td>
</tr>
<tr>
<td>SAS Average Severity</td>
<td>1.6 (1.1)</td>
<td>1.9 (1.3)</td>
<td>.39</td>
</tr>
</tbody>
</table>

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 Neuropsychological Performance

<table>
<thead>
<tr>
<th>Measure</th>
<th>Controls (n=16)</th>
<th>Concussed (n=25)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVLT-II Trials 1-5 T-score</td>
<td>57.0 (11.6)</td>
<td>55.7 (10.4)</td>
<td>.72</td>
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<tr>
<td>CVLT-II Short Delay Z score</td>
<td>0.41 (1.39)</td>
<td>0.36 (1.12)</td>
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<tr>
<td>CVLT-II Long Delay Z score</td>
<td>0.43 (1.25)</td>
<td>0.32 (0.76)</td>
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<td>WMS-IV LM I Scaled Score</td>
<td>10.6 (2.5)</td>
<td>10.7 (2.9)</td>
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<tr>
<td>WMS-IV LM II Scaled Score</td>
<td>10.3 (3.1)</td>
<td>10.5 (2.7)</td>
<td>.83</td>
</tr>
</tbody>
</table>

Note. Values are mean and (SD). CVLT-II = California Verbal Learning Test-II; WMS-IV = Wechsler Memory Scale -IV; LM I = Logical Memory Immediate Recall; LM II = Logical Memory Delayed Recall.
The T-Score distribution has a mean of 50 and a standard deviation of 10.
The Z-score distribution has a mean of 0 and a standard deviation of 1.0
The scaled score distribution has a mean of 10 and a standard deviation of 3.
Table 3.3 Behavioral performance on the verbal memory task

<table>
<thead>
<tr>
<th>Condition</th>
<th>Controls (n=16)</th>
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<tr>
<td>Subjective correct responses (%)</td>
<td>77.6 (13.0)</td>
<td>74.6 (18.9)</td>
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<td>Post-task free recall (%)</td>
<td>53.0 (25.8)</td>
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<tr>
<td>Post-task cued recall (%)</td>
<td>76.8 (23.0)</td>
<td>68.5 (28.1)</td>
<td>.34</td>
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*Note.* Values are mean and (SD)
Table 3.4 Whole-brain fMRI results for the Encoding-View Condition by group

<table>
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<tr>
<th>Cluster</th>
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Note. Threshold for each group was p < .05, FWE corrected, with a minimum count of 8 contiguous voxels. Regions were identified using the AAL and Harvard Cortical/Subcortical Atlases. Coordinates are in MNI space.
# Table 3.5 Whole-brain group comparisons during the Encoding-View condition

<table>
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<tr>
<th>Cluster</th>
<th>Maxima x</th>
<th>y</th>
<th>z</th>
<th>R/L</th>
<th>Region</th>
<th>Extent</th>
<th>Peak T</th>
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Note. Threshold for each group was p < .005, uncorrected, with a minimum count of 10 contiguous voxels. Regions were identified using the AAL and Harvard Cortical/Subcortical Atlases. Coordinates are in MNI space.
Table 3.6 Whole-Brain results for the Recall-View condition by Group.

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<td>Paracingulate/anterior cingulate gyrus</td>
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Note. Threshold for each group was p < .05, FWE corrected, with a minimum count of 8 contiguous voxels. Regions were identified using the AAL and Harvard Cortical/Subcortical Atlases. Coordinates are in MNI space.
Table 3.7 Whole-brain group comparisons during the Recall-View condition

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<td>12</td>
<td>28 L</td>
<td>26</td>
<td>3.38</td>
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</table>

Note. Threshold for each group was p < .005, uncorrected, with a minimum count of 10 contiguous voxels. Regions were identified using the AAL and Harvard Cortical/Subcortical Atlases. Coordinates are in MNI space.
Figure 3.1. Example of the verbal memory task progression. Each experimental condition (Encoding, Distractor, Recall) began with a 3s instruction, followed by the presentation of words or letters. After the presentation of each set of words, a crosshair was presented for 0.9s. Each condition lasted for 19.5s before moving to the next condition.
Figure 3.2. Example of the verbal memory task progression across the experiment. Each experimental condition had a 3s instruction presentation followed by 19.5s of the active task. The Recall block was followed by a crosshair for 6s. These blocked were presented a total of ten times, for a paradigm lasting 12m 24s.
Figure 3.3 Accuracy for the verbal memory paradigm.
Figure 3.4 Single group whole-brain activation pattern for the Encoding-View contrast at the statistical threshold of $p < .05$, family-wise error corrected with at least 8 contiguous voxels.
Figure 3.5 Single group whole-brain activation pattern for the Recall-View contrast at the statistical threshold of $p < .05$, family-wise error corrected with at least 8 contiguous voxels.
Figure 3.6 Brain regions associated with greater activation in the control participants compared to the previously concussed participants during the Encoding-View contrast at the threshold of $p < .005$, with at least 10 contiguous voxels.
CHAPTER 4

DISCUSSION

The current experiment examined verbal memory processes in former high school football players who sustained multiple concussions during their high school playing years to expand knowledge related to the potential long-term effects associated with cumulative head injuries. Like many previous studies, we failed to find differences in performance associated with concussive histories on neuropsychological tasks assessing memory in our two closely matched groups (Dikmen, et al. 1995; Ettenhofer & Abeles, 2009; Echemendia, et al., 2001; Macciocchi, et al. 1996; Carroll et al., 2004). However, we found modest differences in brain activity such that those with a history of multiple concussions have reduced activation in areas associated with language when learning and recalling verbal material. The regions that exhibited hypoactivation during the encoding task have previously been associated with the “levels-of-processing” model of language functioning (Craik & Lockhard, 1972), which posits that encoding more complex information requires additional cortical resources and therefore increases retention. Therefore, concussed individuals may have some underlying changes in their verbal memory encoding system that limits them from accessing higher-order semantic networks. This was further supported by left middle frontal lobe hypoactivation during retrieval, which has previously been implicated in the encoding and retrieval of detailed verbal information (Ranganath, et al. 2000).

These findings do not support our initial hypotheses, which was that those with concussive injuries would have an increased magnitude and extent of activation in memory.
related regions compared to the control group. These hypotheses were based on literature that typically examined concussive injuries in the acute period of recovery (e.g. Dettwiler et al., 2014; Jantzen, et al., 2004; Lovell et al., 2007; McAllister et al., 1999; McAllister et al., 2001; Slobounov et al., 2010; Zhang et al., 2010), which predominantly posited that a history of head injuries is associated with hyperactivation in cortical networks.

However, since the proposal of this study, a newer body of literature has emerged examining the post-acute/chronic changes that may be associated with mTBI. These studies have generally found attenuated BOLD signal as compared to control participants in both symptomatic (Gosselin et al., 2011; Ge et al., 2009) and asymptomatic participants (Ford et al., 2013; Monti, et al., 2013) in task-related cortical networks. The results of the current study are consistent with the two recent studies that have methodological similarities, such that middle-aged individuals with a history of medically diagnosed concussions before the age of 25 showed hypoactivation during memory retrieval in the middle PFC, precentral gyrus, and other regions (Monti, et al., 2013) and that former NFL players with multiple concussions showed hypoactivation in the right parahippocampal gyrus and bilateral inferior parietal cortex (BA 40) in successful retrieval trials compared to the control group (Ford, et al., 2013). Discordant with the current findings, Ford et al. (2013) showed increased activation in the medial PFC whereas the current results show decreased medial PFC activation during the retrieval phase. This difference may be due to methodological differences between the studies given that participants in the Ford et al. (2013) indicated whether they either had or had not seen the word pair previously, introducing potential error monitoring neural networks into the paradigm.

Single group activation patterns in the current study did not show neural recruitment in the hippocampus or parahippocampal regions as expected. The paradigm used has been shown to
recruit memory networks effectively in previous studies (Bookheimer et al., 2000; Braskie et al., 2009; Ford et al., 2013). This could be explained in several ways. Subcortical structures could have been similarly engaged during both the task (i.e. encoding, recall) conditions as well as the control condition, which would have inhibited activation in these regions to be seen in contrast analyses. This could have occurred if participants continued to engage in rehearsal strategies during the “view” task, or if the hemodynamic response of the medial temporal lobe systems took an increased amount of time to return to baseline. Another possibility to explain lack of MTL activation is related to susceptibility artifacts during fMRI image acquisition that make it difficult to detect BOLD signal in anterior MTL regions (Ojemann, Akbudak, Snyder, McKinstry, Raichle, & Conturo, 1997). Further, the signal-to-noise ratio is shown to be higher in MTL regions than several other brain structures (Schacter & Wagner, 1999), which may decrease the ability to capture subtle changes in blood oxygenation.

Exploring these data in various other ways did not yield different or meaningful findings. In our functional imaging analysis, we took a statistically conservative approach by first contrasting each participant’s task related activity with the activity during distractor task (e.g. Encoding-view), and then compared the difference maps across groups (e.g. Control Encoding-view vs. Concussed Encoding-view). This would theoretically yield the differences in task-specific BOLD signal across groups. However, post-hoc, we analyzed the data using a more liberal approach by not taking the distractor task into account. Thus, we compared all of the activation during the task (e.g. Encoding) across the two groups (Controls encoding vs. Concussed encoding). Differences in brain activation were comparable to results reported.

We also examined the data at a more liberal threshold to determine any subtle differences between groups. At the statistical threshold of $p < .01$, uncorrected, 8 contiguous voxels, results
looked predominantly the same as at the most rigorous threshold of $p < .005$, 10 contiguous voxels. Given that significant findings at this threshold have a greater probability being attributed to a Type 1 error, we chose only to report the results at the more rigorous threshold in the manuscript since this threshold has an adequate balance between Type 1 and Type 2 error rates in fMRI data (Lieberman & Cunningham, 2009).

As discussed in Chapter 3, this study has several limitations that should be taken into account when interpreting data, including the self-report nature of concussive histories, convenience sampling methods, and a specialized sample that may limit the generalizability of the findings. This study was also cross-sectional, so it is unclear how the impact of aging or older adulthood may influence the participants in the present study. Related to study design, we used a block design framework, which presents several stimuli that represent the same cognitive construct in short epochs of successive trials. Although this type of design has been shown to have a high signal-to-noise ratio, thus making it more ideal for ROI analyses, it does not allow for the comparison of different trial types within a block (e.g. correct vs. incorrect trials) or take the transient responses at the beginning or end of blocks into account (Dale & Buckner, 1997; Donaldson, Petersen, Ollinger, & Buckner, 2001). Using an event-related design may help differentiate the neural networks related to successful encoding and unsuccessful encoding across groups.

Despite these limitations, this study had many strengths. For instance, we validated concussive histories using an empirically based assessment tool unlike several other studies. We also used this tool to screen out a history of concussions in our control group to ensure they did not have a concussive history. All participants performed adequately on measure assessing task engagement, which suggests the participants were putting forth sufficient effort on the remainder
of testing and were not trying to exaggerate or malinger symptoms as previous studies have warned (Green et al., 1999). We also took extraneous variables into account by excluding those with psychiatric, neurological, and developmental diagnoses and ensuring that our two groups were matched on potentially confounding variables like age, education, and premorbid IQ. Further, both groups reported similar numbers of present symptoms associated with a concussion, suggesting that our findings are not a result of a symptomatic experimental group.

Future neuroimaging investigations are necessary to clarify the specific nature of concussive injuries on brain structure and function. We showed that a history of multiple mTBIs is associated with differences in neural activation during a verbal learning and memory task. Given that neuroimaging is a tool that has the potential to help clinicians detect pathology before the presence of clinical symptoms (Bookheimer, et al., 2000), it would be worth examining whether these differences are present in tasks assessing other cognitive domains (e.g. working memory, language processing, visuospatial). Additionally, investigating resting state activation/default mode network connectivity would be beneficial to help determine if atypical activation patterns are diffuse and present in the absence of task stimuli. It will also be important to determine if these changes in brain activity are representative of underlying structural abnormalities. Examining volumes of brain regions using traditional MRI and white matter integrity using diffusion tensor imaging would prove useful.
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