Proposal for a Thesis

in the Field of Biology

in Partial Fulfillment of Requirements for

the Master of Liberal Arts Degree

Harvard University

Extension School

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Name

Address

Phone number

Email address

I.

Tentative Title

"The relationship between extracellular free-water and gray matter volume in retired professional football players with history of mild repetitive head injuries"

II.

The Research Problem

Increased focus has been given to the prevalence of mild traumatic brain injuries (mTBI), better known as concussions in sports. A concussion occurs when a sudden force inflicted on an individual causes the brain to quickly shift its position in the skull and violently collide with the cranium. Nausea, vomiting, irritability, and headaches are all symptoms of someone who has suffered a mTBI (Koerte et al., 2015). The dangers associated with mTBI have evolved both the rules and procedures that govern various sports, as well as technologies used in equipment to better protect the participant. Athletes, particularly those that participate in high contact sports such as football, boxing, ice hockey, etc. have a noticeably increased probability of experiencing multiple mTBI's over the course of their careers (Omalu, 2014). While most athletes who experience a head injury while playing a sport is minor, recovery time ranges from a few days to weeks. However, a smaller group of individuals develop prolonged symptoms. Evidence also suggests that 17% of athletes who experience multiple concussions or events of head trauma are likely to develop a progressive neurodegenerative disease known as Chronic Traumatic Encephalopathy (CTE) (McKee et al., 2009).

Recently the work of medical imaging, such as magnetic resonance imaging (MRI), has been proven useful for early detection of brain alterations stemming from repetitive head trauma as well as the onset of a neurodegenerative disease (Koerte et al., 2015). Imaging techniques have also been useful in determining the underlying pathological mechanisms in brain injuries as well as the development of neurodegeneration. Additional roles provided by neuroimaging techniques include "a role in the identification of treatable injuries, and prevention of secondary damage" (Bruce et al., 2015). Information provided from neuroimaging can also be used to form long-term treatment to the patient, as well as developing their rehabilitation.

Many neurodegenerative diseases function in similar ways in the brain and in turn have comparable causes. Also CTE develops throughout the lifespan, the individual begins to show symptoms similar to Alzheimer's disease. These symptoms include emotional outbursts, lapses in executive functioning skills, as well as depression and reports of suicidal thoughts (Koerte et al., 2015). Many of the expected pathologies of CTE are also extremely similar to those seen in Alzheimer's disease, although there are also some interesting differences. Both diseases are considered to be taupathies, due to their manipulation of the protein tau. The result of this manipulation is neurofibrillary tangles (NFTs), neuropil threads (NTs) and glial tangles (GTs). However, in CTE the tau pathology is found in the cortical laminae (layers II and III), where in AD it is located in layers III and V. It is also relatively irregular in its location prevalence throughout the brain. In Alzheimer's disease the tau hallmarks are observed to be more uniform (Gavett, Stern, & McKee, 2011) Another major micropathological hallmark of Alzheimer's disease is the alternative processing of beta-amyloid protein. This irregular protein formation is regularly seen in the development of Alzheimer's disease but is only present in approximately 40% of CTE cases (McKee et al., 2009). This distinction demonstrates that while there are many

similarities between the symptoms and pathogenesis between the two disorders they are in turn different.

Prior research has shown the degeneration of white matter in patients with Alzheimer's disease on a microstructural level and its association with gray matter atrophy (Maier-Hein et al., 2015). It is our aim in this project to determine whether or not the pathological similarities found in CTE can show relatable results. Here we will apply similar methods on a experimental group of retired NFL players with a history of repeated head trauma. Finding similarities between these disorders could yield a common solution or methods of treatments for those affected. By monitoring the disease's progression, it is also possible to eventually give an earlier diagnosis and take a more preventative than reactive treatment approach. In contrast, examining the differences that make the disorders so unique may provide better more specific treatment, enhance diagnosis and make the symptoms of each independent disease more beneficial to the patient.

A combination of magnetic resonance imaging (MRI) acquisition and analysis methods makes it possible to determine atrophy of white and gray matter of the brain by providing an accurate estimation of volume measurement. Initially, we will use the FreeSurfer software to conduct volumetric analysis on the amount of brain tissue found in each subject (Fischl & Dale, 2000). This may give a clearer picture to the progression of the disease for each subject. Secondly, we will examine further microstructural changes in the white matter of the brain by using an imaging technique called diffusion MRI (Shenton et al., 2012). This could potentially also discover microstructural precursors to CTE. In a step to uncover abnormalities hidden by extracellular water, in cases such as atrophy or edema caused by neural inflammation, a method known as free-water imaging would then be applied to the images in order to eliminate excess

extracellular contributions not attributed to the brain tissue (Pasternak et al., 2012). Eliminating this fraction of free-water not attributed to the neurons themselves will allow a clearer view of microstructural changes in the white matter. In addition, quantifying the amount of free-water will provide an additional microstructural measure of atrophy, and possibly neuroinflammation. We will then use a program suite known as tract based spatial statistics software (TBSS) (Smith et al., 2006), which uses the information from the diffusion-weighted images (DWI) to perform statistical analysis in order to identify significant microstructural white matter differences between the control and test groups. We will then statistically determine correlations between the volumetric and microstructural information.

III.

Definition of Terms

Anisotropy: A measure of the directional dependence of water diffusion. For example high in white matter, where diffusion follows a specific direction along the fiber.

Amyloid Beta (Aβ) Peptide: A peptide that forms plaques, which are known to drive the pathogenesis of Alzheimer's disease.

Chronic Traumatic Encephalopathy (CTE): A progressive neurodegenerative disease caused by repetitive head trauma. Symptoms associated with CTE include memory impairment, behavioral and personality changes, etc.

Diffusion Tensor Imaging (DTI): A technique to analyze data obtained in Diffusion MRI. One of its important measures is the amount of anisotropy in the white matter tracts of the brain.

Diffusion MRI: MRI sequence that detects and visualizes the movement of intracellular and extracellular water molecules in the brain. The sequence acquires signal from different directions, each provides a diffusion-weighted image (DWI).

Eddy Current: A circular electromagnetic current in the MRI that results with image deformation and is caused by the conductors within the instrument. Corrected for in image processing pipeline.

Echo Planar Image (EPI): An imaging method that collects a slice in a single shot to speed up the acquisition and reduce the chance of movement during an MRI image collection.

Fractional Anisotropy (FA): A measurement between 0 and 1 that displays the directionality dependence of diffusion and can help establish claims of microstructural integrity.

FreeSurfer: A software package that contains a set of tools that are used to visualize and analyze the neural anatomy in MRIs.

Free Water: Extracellular water molecules that do not experience flow and are not restricted by their surroundings. Examples of free water in the brain are cerebrospinal fluid (CSF) and forms of edema, which are caused by brain trauma, tumors, and hemorrhages.

Gray Matter (GM): The main portion of the central nervous system. Appearing darker in color, the gray matter includes a majority of the neuron's cell body.

Immunoexcitotoxicity: The interaction between immune receptors (released during injury) within the central nervous system (CNS) and excitatory glutamate receptors trigger a series of events, which then leads to neuronal damage.

Isotropy: A measure of water diffusion in the white matter in which the diffusion is identical in all directions.

Structural MRI: An MRI image of the brain that is used in order to examine the anatomy of both superficial and deep structures of the brain. Examples include T1 and T2-weighted images.

Tau Protein: A protein that is responsible for creating tracks that move nutrients throughout cells in the body. In neurodegenerative diseases, Tau collapses and forms tangles known as neurofibrillary tangles, which destroy the tracks.

Tract Based Spatial Statistics (TBSS): A software script that uses the fractional anisotropy data gathered from diffusion MRI and aligns multiple subjects into a common white matter skeleton. Images from different subjects (e.g., patients and controls) are then projected onto the skeleton and by using statistical analysis, notable changes between groups in the skeleton can be determined.

Traumatic Brain Injury (TBI): An injury that results from a violent blow to the head region. Mild traumatic brain injury (mTBI) includes but is not limited to concussions.

Voxel: A unit in the MRI image that represents 3 mm cube of brain tissue.

White Matter (WM): Consists of mostly myelinated axons. This portion of the brain matter is used primarily in intercellular communication.

IV.

Background of the Problem

Chronic Traumatic Encephalopathy (CTE), unlike a concussion, is a permanent condition that begins around midlife and progresses throughout the life of the affected individual. CTE , unlike a concussion or mTBI, is considered to be a progressive neurodegenerative disease meaning it worsens over the course of the victims life and dismantles their nervous system (Omalu, 2014). CTE is currently diagnosed post-mortem by the identification of tau pathology

without amyloid beta pathology (McKee et al., 2009). The first symptoms of CTE that are exhibited include a decline in concentration and attention followed by complaints of memory loss and headache (Saulle & Greenwald, 2012). Also during this initial stage of CTE witnesses close to the individual report a prevalence of mood swings and irritability (Omalu, 2014). Emotional distress can be so extreme to eventually lead to long-term depression and higher risk of suicide (Saulle & Greenwald, 2012). The severity of the disorder is related to the amount of years playing the sport and the amount of traumatic head injuries that were sustained during that experience

As with any neurodegenerative disease, including Alzheimer's disease, the pathology of CTE features a significant loss of brain mass. According to McKee et. al. atrophy has an increased effect on the frontal (36% decrease in volume), temporal lobe (31%) and parietal lobe (22%). The decrease in volume of the occipital lobe is less significant (3%) (McKee et al., 2009). Additional gross anatomical evidence of the disorder include a thinning of the hypothalamus (Gavett et al., 2011). The ventricles are enlarged due to the abnormal movement of fluid caused by the multiple instances of head trauma. Other notable areas, according to Gavett 2011, that are significantly decreased include the cerebrum, diencephalon, basal ganglia, brainstem and cerebellum.

On a microscopic level CTE continues to resemble Alzheimer's disease in an array of ways. CTE and Alzheimer's disease are classified as taupathies because of their effect on the protein tau. The tau protein's role in an organism is to construct and stabilize microtubules, which are needed to transport valuable resources throughout the cell (Hanger, Anderton, & Noble, 2009). When the microtubules destabilize it has a cascading effect of starving the cells of the materials it needs, which eventually leads to the destruction of the cells and tissues. Tau

proteins are in high numbers alongside the axon of neurons and in astrocytes and oligodendrocytes used to protect and maintain the neurons. The damage to the tau proteins then leads to the presence of neurofibrillary tangles (NFTs), astrocytic tangles and neuropils (NT) (McKee et al., 2009). According to Saulle et al 2012, areas that show an increased presence of neurofibrillary tangles as well as neuropil threads include but are not limited to the amygdala, the hippocampus, the thalamus, the hypothalamus etc. These are the same hallmarks found in Alzheimer's disease due to its effect of the tau protein. However, one distinction that is known that differs between the two disorders is that in CTE the NFTs are located more in the superficial cortical laminae, whereas in the Alzheimer's pathology the NFTs develop on neurons in deeper layers of the brain. (Saulle & Greenwald, 2012)

There are multiple theories on the pathogenesis of CTE. The first of which is axonal damage due to shearing to the axon that was caused by the trauma the brain (Johnson, Stewart, & Smith, 2010). However, now there is a thought that this axonal shearing is a downstream effect of CTE, and the disease is caused by neuroinflammation and degeneration of the axons. The shearing due to the sudden violent displacement of the cranium repeatedly causes membrane permeability alterations, which would cause an increase in calcium concentration. The body's defense against this would trigger the phosphorylation of tau protein and the accumulation of neurofibrillary tangles and microtubules (Gavett et al., 2011). A paper by Blaylock and Maroon describes the central mechanism of CTE to be immunoexcitotoxicity. This theory consists of the triggering and priming of microglial cells during the time of head trauma events. When this activity occurs on a repeated basis the microglial cells remain excited and begin to secrete chemicals such as cytokines, chemokines, immune mediators, and excitotoxins in an attempt to repair the damage. These excitotoxins then prevent enzymes known as phosphatases, which are

responsible partially for the regulation of tau protein. This behavior would then encourage the hyperphosphorylation of tau that leads to the formation of neurofibrillary tangles (Blaylock & Maroon, 2011). It is these observable links found in both diseases that promote the line of questions that perhaps the two diseases could show similar results to prevention techniques and treatment options.

The second hallmark that is shared between the two neurodegenerative diseases includes the formation of beta-amyloid plaques. According to the beta-amyloid hypothesis, the alternative cleaving of the amyloid precursor protein is the main reason between the pathogenesis of Alzheimer's disease (Hardy & Selkoe, 2002). Beta amyloid plaques are found in only 40-45% of individuals with CTE and they when they are present they are shown to be less dense and less uniform when compared to their presence in Alzheimer's disease (Gavett et al., 2011). Based on statistics it seems as though the production and accumulation of the beta-amyloid protein seems to be less to blame for the occurrence of CTE. Even though it plays a minor role it has been shown that head injuries cause an increase in the production of amyloid precursor protein. The up regulation of this protein's production leads to an increase of alternative cleaving and the increased concentration of plaques (Gentleman, Nash, Sweeting, Graham, & Roberts, 1993)

A secondary pathology associated with CTE is the presence trans-activator regulatory DNA-binding protein 43 (TDP-43). TDP-43 plays a significant role in response to axonal injuries located in the central nervous system (Saulle & Greenwald, 2012). A study conducted by McKee et. al. has detected the widespread presence of TDP-43 in about 80% of their CTE cases.

Genotyping of the gene Apolipoprotein E (ApoE) has rendered significant results in the development of neurodegenerative diseases including Alzheimer's disease as well as CTE. Historically, ApoE has been linked with the prognosis of Alzheimer's disease, however in studies it has had an even greater influence on subjects with head trauma (McKee et al., 2009). Both the ApoE3 and the ApoE4 genotype were examined in multiple studies and have proven to effect cognition in populations with a history of brain trauma. In mouse models, the ApoE4 allele has had an increased mortality rate than those ApoE3 genotype (Saulle & Greenwald, 2012).

The presence of both hallmarks (amyloid plaques and neurofibrillary tangles) as well as behavioral symptoms (memory loss, depression, emotional outburst, etc.) are observed in both disorders leads to a belief that the two disorders could be related. The occurrence of neurofibrillary tangles caused by tau protein and the plaques in some cases caused by beta-amyloid, occurs within the white matter and the volumetric atrophy was noted in the gray matter. The results of the work conducted by Maier-Hein et al. showed a correlation between the amounts of free-water detected in the white matter and the volume of gray matter. They found that when gray matter volume decreased, there was an increase of free water. By using free-water analysis correctional techniques Maier-Hein et. al. determined that atrophy occurred in both white matter and gray matter, but was effected extracellular volume and density of white matter, which was not accounted for in the white matter volume measure (Maier-Hein et al., 2015). By showing the correlation between free water and gray matter in Alzheimer's disease the research team was able to hypothesize that there should be a shared mechanism that caused both effects. Our aim is to determine whether or not these results were reproduced in a sample population of individuals with a history of head trauma.

The purpose for our experiments is two-fold. Firstly, we aim to determine whether this correlation is reproducible in a population of retired football players with a history of repeated head trauma. Secondly, we aim to determine whether free-water correction techniques can provide insight to total brain matter atrophy before a diagnosis of CTE.

V.

Research Methods

This study will investigate the relationship between free-water in the white matter and gray matter volume in retired professional football players with history of mild repetitive head injuries. Brigham and Women's Hospital located in Boston, Massachusetts, collected the images used in this study. Data will be processed and analyzed in the Brigham and Women's Psychiatry Neuroimaging Laboratory under the supervision of Dr. Ofer Pasternak.

Magnetic Resonance Imaging (MRI) is an imaging technique that creates images of body structures by using strong magnetic fields and radio waves. This technique utilizes the hydrogen atoms that are abundant in the body in molecules such as carbohydrates, fats, and most commonly water. MRI applies a strong magnetic field that aligns the direction of the hydrogen atoms precession. Then by radio waves in specific frequencies are used to change the direction of precession. During the return of the molecules to the main magnetic field direction, energy is emitted, which is used to generate an image (Susumu Mori, 2007). The color intensity of the image pixels corresponds to the concentration of protons (usually from hydrogen atoms of water) in the area (S. Mori & Zhang, 2006). In the purpose of our study we used a combination of structural and diffusion MRI techniques.

FreeSurfer is a program that was developed at the Martino's Center for Biomedical Imaging at Massachusetts General Hospital in Boston, Massachusetts that estimates volumes and shapes of different brain parts from structural brain images. These images are referred to as T1 or T2 weighted images and show richer qualitative and quantitative details of the brain than respective diffusion MRI technique. FreeSurfer software has a variety of uses including volumetric segmentation, segmentation of white matter, parcellation of cortical folding patterns, mapping the thickness of cortical grey matter, and the construction of surface models of the cerebral context (Fischl, 2012). For our purposes we will use FreeSurfer as a measurement tool in order to determine the volume of the white and gray matter of the brain. Through a process called segmentation, "information including the statistical properties of the anatomical structures are stored in a space where their coordinates possess anatomical meaning, as opposed to arbitrary coordinates found in the raw structural image" (Fischl et al., 2002). The image is composed of small cubical sections known as voxels, where all of the information is stored. Each voxel is assigned characteristics such as whether it belongs to white matter, gray matter or cerebrospinal fluid (CSF). Those voxels are then clustered based on brain tissue type. For example, gray matter is clustered together and would be segregated from white matter voxel clusters. This whole brain segmentation and clustering procedure allows programs such as FreeSurfer to quantify the amount of white matter, gray matter, CSF, as well as the volume of specific brain structures.

An important imaging technique used when examining neural anatomy both on a gross and on a microstructural level is diffusion MRI. Diffusion MRI quantifies the random movement of water molecules (or Brownian Motion) located within the brain tissue. The diffusion of water is not unique to white matter tracts but can be observed in any other tissue, like kidney, skeletal muscle, and cardiac muscle. However the degree of diffusion anisotropy in

tissues besides white matter tracts seem to be much less significant (Beaulieu, 2002). Images produced from a diffusion MRI scan are known as diffusion weighted images (DWI). Every voxel in the DWI is colored black, white or an intermediate shade of gray. The voxel color correlates to the amount of water diffusion at that site, and along a specified direction. Combining the information from several directions can be used to derive indicators of tissue microstructure.

DTI is a model that is utilized in order to analyze and to quantify the diffusion properties of the white matter bundles (Assaf & Pasternak, 2008). The DTI model is able to determine the three-dimensional diffusion profile in which water molecules move throughout brain tissue. The importance of assigning a quantity to the DWI allows us to compare data between groups of subjects and also to draw conclusions based on the biological processes occurring in the brain tissue. DTI uses the movement of water to make inferences about the neural anatomy (S. Mori & Zhang, 2006). For example, in the axons of neurons water perpendicular to the axon is mostly restricted from movement, but is relatively free to diffuse along the axon. This is known as anisotropic diffusion, meaning that diffusion is directionally dependent (Beaulieu, 2002). This suggests a bundle of fibers that are elongated in the direction the water is diffusing faster. When the diffusion is equal in multiple directions similar to a circle, it is said to be isotropic.

Fractional Anisotropy (FA) is one of the most widely used measures of anisotropic diffusion, which is useful to determine whether the diffusion in that area is isotropic or anisotropic. If the white matter tract is more anisotropic it will assign a higher FA value, closer to 1. In contrast, if indicated the fiber displays more isotropic diffusion the FA will have a lower value closer to 0.

DTI studies have revolutionized the way in which the brain is studied. Applications in research include using anisotropy as a marker for pathological studies, monitoring anisotropy during brain development, gathering information about the myelination of axons, examining the parcellation of white matter, etc. (S. Mori & Zhang, 2006). One limitation to diffusion MRI and the quantification of anisotropy is when water is accumulated naturally such as the presence of cerebrospinal fluid (CSF) or inflammatory responses and edema. Manually subtracting out those artifacts proved problematic. Papadakis et. al. conducted a study in which they wanted to suppress the presence of CSF in the MRI image in order to study anisotropy in a normal human brain. They suggested using a technique called fluid-attenuated inversion recovery (FLAIR) that was administered as a preparatory technique that decreased the signal in the image that was caused by CSF (Papadakis et al., 2002). This technique evolved into another correctional technique known as free water analysis (FW) that was able to eliminate the effect of CSF as well as other artifacts such as inflammation, edema and other forms of extracellular water. Free water analysis focuses on water molecules that do not experience flow and that movement is not prohibited due to its surrounding structures (Pasternak, Sochen, Gur, Intrator, & Assaf, 2009). Cerebrospinal fluid (CSF) is an example of free-water in the brain. According to Corsellis et al, one of the most apparent gross pathological findings in CTE is the enlargement of the lateral and third ventricles (Saulle & Greenwald, 2012). The ventricles serve as pumps in the brain in order to carry CSF from the inferior to superior regions of the brain. CSF continuously bathes the brain and it serves to provide a cushion to minimize impact from any physical trauma. Free water can be detected on DTI because it shows isotropic diffusion and can interfere with the diffusion values (Pasternak et al., 2009). Therefore it is of interest to eliminate the effect of free water when quantifying diffusion in tissue. Edema and tumors provide similar contamination to DTI

that is shown with CSF contamination. Additional areas that show an increase in CSF contamination for DTI include the fornix, the cingulum and the corpus callosum (Papadakis et al., 2002). All of these structures are in close proximity to the ventricles used to transport CSF.

Subjects that have experienced repeated instances of head trauma have been observed to contain excess edema. In order to extract as much information as possible from the damaged brain a technique can be used to account for the excess free-water detected in the DWI. This correctional technique is commonly referred to as free-water imaging (Pasternak et al., 2009). Free water imaging accurately detects and subtracts out the component of free-water that appears in the extracellular space, and could be affected by the presence of hemorrhaging, brain tumors, pooling of cerebrospinal fluid, and edema caused by trauma to the brain.

In order to determine any microstructural changes in the scans, statistics must be collected. The images are then compiled through a statistics software package called tract-based spatial statistics (TBSS) (Smith et al., 2006). TBSS can examine FA as well as other modalities derived from the DTI. These include FAt, radial diffusivity (RDt), and axial diffusivity (ADt). All of the following modalities provide data corrected for the presence of free-water. Both ADt and RDt are derived from the parallel and perpendicular routes water can diffuse along the axon's fibers. These are additional measures to detect potential white matter deficits. TBSS projects the data received from the diffusion image and projects it onto a white matter skeleton that includes the fractional anisotropy means. Downstream, an additional script called ENIGMA that divides the image voxel by voxel into different regions of interest that can be used to draw conclusions about specific structures of the brain.

The study will focus on one experimental sample set consisting of retired professional football players with a history of head trauma as well as controls that have no experiences of

head trauma. Each individual participating in the study was imaged and that is stored in the psychiatric neuroimaging laboratory. For the purpose of this project we plan to perform the following analyses: First, the images would go through preprocessing and quality control steps that align and center the images in order to ready them for downstream processing. The images will then be masked either manually (diffusion MRI) or automated (structural MRI). Masking is a process in which the user or computer determines which part of the MR image correspond with brain of the subject, and which is non-brain (skull, shadow, artifact, etc.). After this step the structural image would then be run through the FreeSurfer program for segmentation and parcellation of the brain. The software is responsible for dividing the brain into its corresponding structures based on the image.

Diffusion analysis will be performed on the diffusion images. The analysis focuses on creating a skeleton or template of the white matter tracts in the brain. It will utilize a combination of two software packages to achieve this. Firstly it will use TBSS in order to create white matter skeleton based on information contained within the image. Further downstream, we will use a protocol called ENIGMA in order to provide regions of interest to correspond to the white matter skeleton created by TBSS. The information ENIGMA collects allows the user to observe and draw conclusions from the diffusion located at specific regions of the brain. Using the volumetric and diffusion data extracted from the image results we would then be able to determine whether or not there was a correlation present between the amount of gray matter and the integrity of the white matter. Additional correlations between specific regions of interest of the brain will be conducted to see if there is a relationship between the free water amounts and gray matter volume. TBSS will be used to compare control and experimental group. Group comparisons will be conducted separately for each modality (both free water corrected and raw scans). Pearson

correlation test (r) with a significance threshold of (P=0.05) will be used to indicate the potential association between gray matter volumes and amount of free water. Group differences between volume of gray matter and free water measures will be conducted by using a standard t-test to detect whether or not the experimental group of retired NFL players with a history of mTBI contains statistically significant differences from the control group of subjects with no history of mTBI.

VI.

Research Limitations

With diseases such as Alzheimer's disease and Chronic Traumatic Encephalopathy (CTE), while the patient may show multiple symptoms associated with the disease, a definite diagnosis cannot be made until post-mortem. We expect our experimental group of retired athletes to be comparable to subjects with CTE due to their history of trauma to the head. The subjects could also develop stages of brain atrophy, which would skew the results for correlation.

Technical limitations can occur within the MR image collection process such as movement, which results in a ghosting of an image. Although this more frequently presents a problem with a younger sample set it still can occur and render exclusions in the data collected. Motion artifacts are imminent, unless the subject is anesthetized. To reduce the effect of noise, we will apply noise correction procedures that align the images taken at different times. We will also quantify the amount of motion and include it as a covariate in our statistical analysis to prevent from bias, incase one of the group moves on average more than the other. Another major artifact that is prominent in diffusion imaging is the presence of eddy currents. Eddy currents occur with the magnetic gradient is changed suddenly and may cause a magnetic field gradient to prolong even after the switch the MRI scanner is switched off (Le Bihan, Poupon, Amadon, &

Lethimonnier, 2006). The PNL laboratory in association with Brigham and Women's Hospital uses scripts that were generated in order to correct for the eddy current artifacts in order to optimize their image results.

The groups of subjects determined for this paper also possess threats to validity to the study. As noted earlier, a diagnosis of CTE cannot be made until postmortem. Therefore it is very difficult to be able to draw conclusions about the pathology of CTE, if the subjects used in the study do not have a confirmed diagnosis of the disease. However, because these subjects have a history that includes a high frequency of mTBIs the correlations they yield would address the effect of brain trauma on atrophy and tissue damage. Additionally, the control subject were screened to ensure that they did not have a history of mTBI, but were not genetically screened for genetic factors of neurodegenerative disorders such as the homozygous ApoE4 allele that leads to a significant increase in the development of both Alzheimer's disease or CTE. (Saulle & Greenwald, 2012)

Atrophy of tissue is a process that occurs during the aging process. Therefore volumetric changes in the gray matter of the control group are expected based on age of subject. However, it is our hypothesis that there would be increased atrophy in the retired NFL player population due to the prevalence of mTBI.

An additional limitation is that we might be unable to determine a correlation between the amount of gray matter and free water. If a correlation is not determined it shows us that the method the brain atrophies is different between Alzheimer's disease and CTE are different. By understanding the similarities and differences between the two neurodegenerative diseases it can provide a clearer direction for diagnosis as well as both preventative and reactive treatment options.

VII.

Tentative Schedule

Submission of proposal to research advisor	December 2015
Proposal returned for revision	January 2016
Submission of final proposal	March 2016
Proposal accepted by research advisor	April 2016
Thesis director agrees to serve	April 2016
First draft returned by thesis director	June 2016
Revised draft completed	June 2016
Revised draft returned by thesis director	July 2016
Final text submitted to thesis director and research advisor	August 2016
Final text approved	August 2016
Bound copies delivered to Extension School	September 2016
Graduation	November 2016

VIII.
References

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- Excellent review of the benefits and methodolgy behind Diffusion Tensor Imaging.
- Examines DTI as a tool to provide information regarding the white matter architecture of the brain.

Beaulieu, C. (2002). The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed, 15*(7-8), 435-455.

- Discusses the use for water diffusion's ansiotrophy as a measurement used by DTI in order to provide information regarding the microstructure of nerve fibers.

Blaylock, R. L., & Maroon, J. (2011). Immunoexcitotoxicity as a central mechanism in chronic traumatic encephalopathy-A unifying hypothesis. *Surg Neurol Int, 2*, 107.

- Explores the hypothesis that a reaction of the immune system after head injury can trigger a series of events that lead to the dendritic retraction, synaptic injury, microtubule damage, etc.
- Links the immunoexcitoxictity reaction to CTE by its hyperphosphorylation of tau protein.

Bruce, E. D., Konda, S., Dean, D. D., Wang, E. W., Huang, J. H., & Little, D. M. (2015). Neuroimaging and traumatic brain injury: State of the field and voids in translational knowledge. *Mol Cell Neurosci, 66*(Pt B), 103-113.

- Discusses the use of neuroimaging techniques as a biomarker that can be used to diagnosed damage inflicted by traumatic brain injury (TBI).
- Reviews the current methods and limitations to those methods of neuroimaging as a biomarker for TBI.

Fischl, B. (2012). FreeSurfer. *Neuroimage, 62*(2), 774-781.

- FreeSurfer is a set of tools that analyzes neuroimaging data and can provide information regarding the functional and structural properties of the brain.
- FreeSurfer is used to provide functions that include whole brain segmentation, thickness estimation, as well as volumetric analysis.

Fischl, B., & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A, 97*(20), 11050-11055.

- Utilized both manual and automated methods for measuring the thickness of the cerebral cortex.
- Cortex provided a irregular shape and made manually measuring thickness very problematic due to many folds in its structure.
- Automated technique, FreeSurfer software package, proved to be a more accurate and reliable method for measuring cortical thickness.

Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., . . . Dale, A. M. (2002). Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron, 33*(3), 341-355.

- Provides an automated technique that labels each voxel of a MRI image to a specific region of the brain. This process is known as segmentation.
- Compared new automated technique against manual technique that was only able to segment image into 26 regions.

Gavett, B. E., Stern, R. A., & McKee, A. C. (2011). Chronic traumatic encephalopathy: a potential late effect of sport-related concussive and subconcussive head trauma. *Clin Sports Med, 30*(1), 179-188, xi.

- Discusses the clinical signs and symptoms of Chronic Traumatic Encephalopathy.
- Reports of microscopic neuropathology including tau hyperphosphorylation, beta-amyloid deposition, TDP-43, etc.

Gentleman, S. M., Nash, M. J., Sweeting, C. J., Graham, D. I., & Roberts, G. W. (1993). Beta-amyloid precursor protein (beta APP) as a marker for axonal injury after head injury. *Neurosci Lett, 160*(2), 139-144.

- Explore the hypothesis that beta-amyloid protein, which is associated with Alzheimer's Disease, is additionally present in the victims who have undergone head injuries.
- Beta-amyloid precursor protein is a excellent biomarker for axonal injury.
- Beta-amyloid precursor protein and beta-amyloid protein are not located near the site of axonal injury.

Hanger, D. P., Anderton, B. H., & Noble, W. (2009). Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. *Trends Mol Med, 15*(3), 112-119.

- Tau is a protein used to stabilize cellular microtubules. Diseases in which tau is effected include but are not limited to Alzheimer's disease and Chronic Traumatic Encephalopathy.
- Hyperphosphorylation of tau protein lead to neuronal death relatively early in the conception of the disease.
- Process of tau hyperphosphorylation is achieved by multiple enzymes that include kinases and phosphotases.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science, 297*(5580), 353-356.

- Examines pathogenesis of Alzheimer's disease that includes the formation and deposition of amyloid beta protein that leads to the formation of plaques.
- Propose therapeutic strategies that combat the effects of amyloid pathogenesis of Alzheimer's disease. This strategy is effective but does not account for tau pathogenesis.

Johnson, V. E., Stewart, W., & Smith, D. H. (2010). Traumatic brain injury and amyloid-beta pathology: a link to Alzheimer's disease? *Nat Rev Neurosci, 11*(5), 361-370.

- Connects the beta-amyloid pathology of Alzheimer's diease to traumatic brain injurys, or physical trauma to the brain.
- States that axonal injury can lead to premature formation of beta-amyloid protein. Protein forms later in Alzherimer's disease, but at time of trauma in TBI.

Koerte, I. K., Lin, A. P., Willems, A., Muehlmann, M., Hufschmidt, J., Coleman, M. J., . . . Shenton, M. E. (2015). A review of neuroimaging findings in repetitive brain trauma. *Brain Pathol, 25*(3), 318-349.

- Explores different types of imaging techniques used for examining repetitive brain trauma. (includes diffusion tensor imaging, positron emission topography and magnetic resonance spectroscopy)
- Describes that the identification of a specific marker of tissue structure can lead to identification of the underlying mechanisms of CTE.

Le Bihan, D., Poupon, C., Amadon, A., & Lethimonnier, F. (2006). Artifacts and pitfalls in diffusion MRI. *Journal of magnetic resonance imaging, 24*(3), 478-488.

- Addresses limitations associated with the technique of Diffusion MRI techniques.
- Describes MRI artifacts such as Eddy Current, motion artificats, ghosting, and EPI artifacts and techniques used to overcome them.

- Echo Planar Imaging is a technique used in biological imaging that takes a single shot to try to prevent motion artifacts.

Maier-Hein, K. H., Westin, C. F., Shenton, M. E., Weiner, M. W., Raj, A., Thomann, P., . . . Pasternak, O. (2015). Widespread white matter degeneration preceding the onset of dementia. *Alzheimers Dement, 11*(5), 485-493 e482.

- Determined a negatively correlation between the FW values and the gray matter volumes in patients that exhibited symptoms of mild cognitive impairment.
- These results suggested atrophy of both the gray and white matter.
- Development of Alzheimer's disease is associated with the white matter abnormalitlies. This result was found in both groups that developed dementia as well as the group that did not.

McKee, A. C., Cantu, R. C., Nowinski, C. J., Hedley-Whyte, E. T., Gavett, B. E., Budson, A. E., . . . Stern, R. A. (2009). Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol, 68*(7), 709-735.

- Explores differences in CTE dependent on different activity the individual participated. (American football, boxers, soccer, and wreslting)
- Discusses volumetric changes in all cases, as well as the presence of neurofibrillary tangles, and beta-amyloid protein deposition.
- Beta-amyloid deposition only found in approximately 40% of CTE cases.

Mori, S. (2007). *Introduction to diffusion tensor imaging*: Elsevier.

- Provides information regarding the physics of magnetic resonance imaging, a non-invasive techniques to study brain disorders and neurdegenerative disease.
- Describes the benefits of diffusion MRI and methods used to measure diffusion.

Mori, S., & Zhang, J. (2006). Principles of diffusion tensor imaging and its applications to basic neuroscience research. *Neuron, 51*(5), 527-539.

- Explains the principles of the diffusion tensor imaging technique.
- Provides insight to the visualization of the axonal tracts of a human and mouse model using DTI.

Omalu, B. (2014). Chronic traumatic encephalopathy. *Prog Neurol Surg, 28*, 38-49.

- Examines the symptoms ranging from effects of TBI to more dementia-like symptoms including memory loss and emotional swings.
- Determines proteinopathies (tau and amyloid-beta) to be end stage pathology of CTE. Believes that future research should try to explore other options that are more proactive for treatment options.

Papadakis, N. G., Martin, K. M., Mustafa, M. H., Wilkinson, I. D., Griffiths, P. D., Huang, C. L., & Woodruff, P. W. (2002). Study of the effect of CSF suppression on white matter diffusion anisotropy mapping of healthy human brain. *Magn Reson Med, 48*(2), 394-398.

- The presence of cerebrospinal fluid may compromise the results of diffusion MRI because it contains a large amount of water detected by this technique.
- Authors compared DTI analysis with a fluid-attenuated inversion recovery technique (FLAIR). The FLAIR post-processing technique focuses on correcting the images for the amount of extracellular water.

Pasternak, O., Sochen, N., Gur, Y., Intrator, N., & Assaf, Y. (2009). Free water elimination and mapping from diffusion MRI. *Magn Reson Med, 62*(3), 717-730.

- Authors propose a method for correcting images that contained excess water (provided from CSF or edema). This method is known as Free Water analysis.
- The volume of free water also served as a quantitative tool. By mapping the volume of free water per voxel one can use as a comparison between groups.

Pasternak, O., Westin, C. F., Bouix, S., Seidman, L. J., Goldstein, J. M., Woo, T. U., . . . Kubicki, M.
(2012). Excessive extracellular volume reveals a neurodegenerative pattern in schizophrenia onset. *J Neurosci, 32*(48), 17365-17372.

- Free water methodology was used on a population of subjects that were diagnosed with schizophrenia and had at least one psychotic episode.
- The research team found a significant increase in both white and gray matter which determined neuroinflammation was a prominent factor in the onset of the disorder.

Saulle, M., & Greenwald, B. D. (2012). Chronic traumatic encephalopathy: a review. *Rehabil Res Pract, 2012*, 816069.

- Comprehensive review of Chronic Traumatic Encephalopathy that includes its gross and microscopic pathology.
- Examines the potential correlation between head trauma and the future development of amyotrophic lateral sclerosis (ALS), or Lou Gehrig's Disease.

- Discusses the genetic factors for CTE including the apolipoprotein E (ApoE). It is noted that if someone is homeozygous for the ApoE4 allele then they have a 14-fold increase of developing Alzheimer's disease.

Shenton, M. E., Hamoda, H. M., Schneiderman, J. S., Bouix, S., Pasternak, O., Rathi, Y., . . . Zafonte, R. (2012). A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imaging Behav, 6*(2), 137-192.

- Investigates the use of MRI and CT imaging techniques in studying mild traumatic brain injuries (mTBI), commonly known as concussions.

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., . . . Behrens, T. E. (2006). Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *Neuroimage, 31*(4), 1487-1505.

- Examines the problem in neuroimaging that it is difficult to align all diffusion images from sample populations and groups in order to draw comparisions and conclusions.
- Tract Based Spatial Statistics (TBSS) software package aims to register all of the images so that these comparisons can be made. TBSS also aims project these images onto a mean FA skeleton.