

South Dakota State University

## Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange

---

Biology and Microbiology Graduate Students  
Plan B Research Projects

Department of Biology and Microbiology

---

2019

### Pathogenesis of CTE: A Review of PTau, Lewy Bodies, and Cytokine Involvement in CTE

Karter Keefer  
*South Dakota State University*

Follow this and additional works at: [https://openprairie.sdstate.edu/biomicro\\_plan-b](https://openprairie.sdstate.edu/biomicro_plan-b)



Part of the [Biology Commons](#), [Microbiology Commons](#), and the [Sports Sciences Commons](#)

---

#### Recommended Citation

Keefer, Karter, "Pathogenesis of CTE: A Review of PTau, Lewy Bodies, and Cytokine Involvement in CTE" (2019). *Biology and Microbiology Graduate Students Plan B Research Projects*. 11.  
[https://openprairie.sdstate.edu/biomicro\\_plan-b/11](https://openprairie.sdstate.edu/biomicro_plan-b/11)

This Plan B - Open Access is brought to you for free and open access by the Department of Biology and Microbiology at Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. It has been accepted for inclusion in Biology and Microbiology Graduate Students Plan B Research Projects by an authorized administrator of Open PRAIRIE: Open Public Research Access Institutional Repository and Information Exchange. For more information, please contact [michael.biondo@sdstate.edu](mailto:michael.biondo@sdstate.edu).

# **Pathogenesis of CTE: A Review of PTau, Lewy Bodies, and Cytokine Involvement in CTE**

**Author: Karter Keefer**

**External Reviewer: Dr. Elizabeth Droke**

## Table of Contents

Pathogenesis of CTE: A Review of PTau, Lewy Bodies, and Cytokine Involvement in CTE.....	1
Abstract .....	3
Main Text .....	4
Clinical Presentation .....	5
Pathogenesis .....	5
Tau.....	5
Lewy Bodies.....	8
Anatomy .....	10
Signs and Symptoms of CTE.....	14
Depression.....	14
Cytokine Regulation .....	15
Behavioral Changes.....	21
Memory Loss.....	22
Diagnosis .....	23
Treatment.....	28
Conclusion .....	29
Limitations.....	29
Future Research.....	29
References and Notes.....	31
Acknowledgements .....	35

**Abstract:**

Chronic Traumatic Encephalopathy (CTE) is a neuropathological disease that develops years after experiencing repetitive traumatic brain injuries. In recent years, this disease has been gaining recognition from professional, college, and high school teams that participate in contact sports. The purpose of this paper is to review available information about this neurodegenerative disease. Only limited published experiments are available on this disease. Research on CTE has been limited by the fact that patients can only be diagnosed with the disease by a post mortem autopsy. Many experiments were analyzed regarding different CTE pathologies, symptoms, and treatments. Articles used in this paper were restricted to only peer reviewed pieces. The outcome resulted in credible information about the disease, its neuropathy, and signs and symptoms, as well as possible treatment methods of the disease.

**Main Text:**

On July 25<sup>th</sup>, 2017 the New York *Times* published data from a study that stated 110 out of 111 former NFL players had been discovered to have Chronic Traumatic Encephalopathy. Chronic Traumatic Encephalopathy, or CTE, is a neurodegenerative disease that has been linked to repetitive, mild traumatic brain injuries. CTE has been associated with a variety of symptoms, some of them being depression, memory loss, and aggression. Signs and symptoms do not appear until years after the initial injury and the disease is very progressive in later years. Understanding CTE first takes an understanding of a traumatic brain injury. A traumatic brain injury (TBI) is a significant blow or jolt to the head that can cause altered brain function or feeling. An example of a mild traumatic brain injury is a concussion. Concussions are a common head injury among participants in contact sports or of those who serve in the military. According to the University of Pittsburgh's Brain Trauma Research Center, there are 300,000 sports-related concussions that occur annually in the U.S. Furthermore, the probability of a participant experiencing a concussion per season is as high as 19% (1). This paper will discuss and review findings that relate these mild traumatic brain injuries (mTBI) and the pathogenesis of CTE.

CTE hasn't always been highly acknowledged in past years. It was initially recognized by Dr. Martland in boxers who suffered from similar symptoms of being classified as "Punch Drunk" (5). Dr. Martland had a career as a medical examiner, forensic pathologist, and teacher in New Jersey and New York (24). Later, Dr. Millspaugh, a naval medical officer, introduced the term "dementia pugilistica," in 1937, to describe these same symptoms (6). It wasn't until 1949, however, that the term "chronic traumatic encephalopathy" was suggested by Dr. Critchley,

world renowned neurologist, and then changed to “chronic *progressive* traumatic encephalopathy of boxers” in 1957 (13,14,15). These sub-concussive hits being suffered by boxers were soon recognized in other contact sport participants and serving military members. All of these sports or activities could result in collisions or concussive blows to the skull that would possibly result in a mild traumatic brain injury (mTBI).

### **Clinical Presentation:**

Previously, brain injuries could only be diagnosed by the patients’ symptom description, which presented 8-10 years after experiencing the mTBI. Symptoms of CTE can include headaches, rapid mood swings, impulsive aggression, increased irritability, aggression, memory loss, varying levels of depression, and, in some cases, gait abnormalities. One of the most severe symptoms can be suicidal thoughts and actions (16).

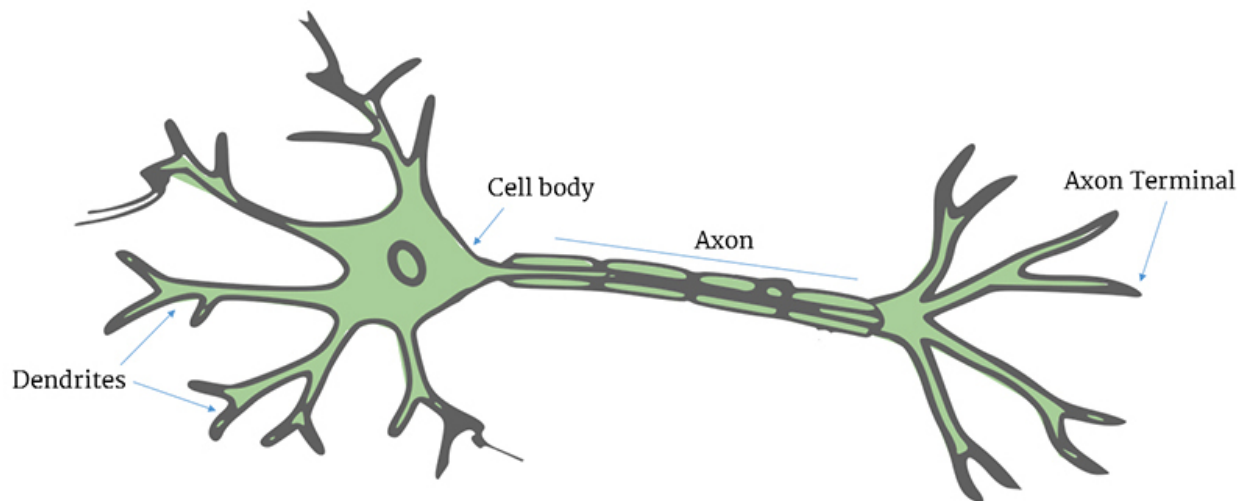
Diagnosing and researching a brain disease or injury such as CTE was historically difficult to do because the only way to see the brain was post mortem. Although the histological techniques varied, the most common pathological findings were cerebral atrophy, neuronal loss, gliosis and argyrophilic neurofibrillary tangles (2). Fortunately, the way we view the brain and possible brain injuries has advanced dramatically over recent years. X-Rays, CT scans, and MRI’s are now used to take images of the brain, and PET scans can even show functions of the brain.

### **Pathogenesis:**

#### *Tau:*

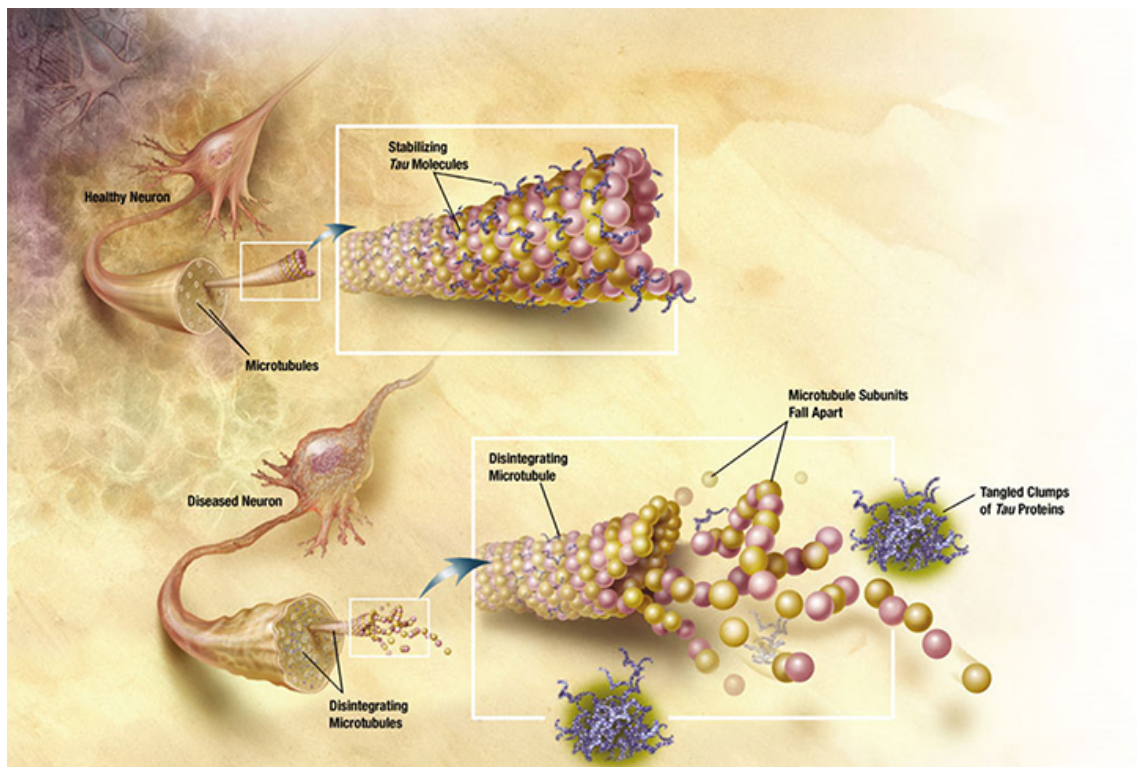
Tau is an abundant protein found in neurons that associates with the stabilization of microtubules and axonal transport (Figure 1) (29). In CTE, specifically, it presents itself in abundance in a unique pattern of neurofibrillary tangles, classified as a taupathy. A taupathy is

any neurodegenerative disorder characterized by abnormally hyper-phosphorylated tau that has misfolded into insoluble aggregates known as neurofibrillary tangles (NFT) (29,2,22). Some examples of common taupathies are progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease, and frontotemporal dementias with parkinsonism and Alzheimer's disease (22).



**Figure 1: A brain cell or neuron.** The axon of the cell is very long and thin, which makes it the weakest point in the cell. Also it is easily damaged during this and can lead to trouble sending signals in the brain (41).

Once hyper-phosphorylated, tau assumes a tightly folded confirmation this causes an increase in susceptibility to aggregation, conformational change, filament assembly, polymerization into paired helical filaments, and bundling of paired helical filaments into neurofibrillary tangles and neuropil threads (Figure 2) (17). Tau phosphorylation also leads to decreased binding with microtubules and lowers overall microtubule stability. The accumulation of tau within these neurons causes synaptic dysfunction and, eventually, cell death (Gou). Studies have shown an association with mutations of the tau gene that lead to dementia and parkinsonism, showing us that a dysfunction of tau proteins is sufficient to cause neurodegeneration and dementia (30).



**Figure 2: A small transport system within the cells is made up of microtubules.** Tau protein helps stabilize these small tubes in a normal healthy brain. In CTE, tau proteins begin clumping to one another and block this transport system (41).

Even though all verified cases of CTE have been related to repetitive mTBI, the mechanism of neurodegeneration and taupathy are not fully understood (2). Previous studies have shown that rapid acceleration-deceleration injuries have been associated with generating these neurotoxic tau fragments, but the development of deterioration has yet to be discovered (2). Symptoms of CTE normally present themselves later in life, which may be due to the prion-like mechanism of p-tau. One theory behind CTE being progressive and is that p-tau can spread and aggregate in other areas of the brain. This theory has been demonstrated in mouse models by injecting areas of their brain with filamentous human Alzheimer's disease linked taupathy proteins and observing their propagation (30). The taupathies expanded from the injection site to other distant brain regions connected by synapses. An additional study confirmed tau



propagation using a silver staining technique to identify tau in neighboring brain regions of hippocampal injected mice (30). The mechanism in which insoluble tau becomes seeded into neighboring cells is absorptive endocytosis. This mechanism was demonstrated in a particular study demonstrating the uptake of a preformed tau fibril by endocytosis recruits large amounts of endogenous tau within the cell to form filamentous tau inclusions (31).

#### *Lewy Bodies:*

Lewy bodies are accumulations of the protein alpha-synuclein within neurons of the brain (38). These accumulations have been associated with Parkinson's disease, Alzheimer's disease, and Lewy body disease (18). CTE, being known as a comorbid disease, has spurred researchers to begin studying these protein accumulations as a possible cofactor of the disease. A recent study sought out a connection between Lewy bodies and CTE within individuals who had suffered mTBI. In this study, researchers took a pool of 694 autopsy participants from 3 distinct brain donation groups and neuro-pathologically evaluated them for neurodegenerative diseases (18). Out of the 694 donations, 269 of the participants were recruited from the Understanding Neurologic Injury and Traumatic Encephalopathy (UNITE) study donation group who had a history of some form of contact sport at the professional or amateur level. The other groups were from the Boston University's Alzheimer Disease Center (ADC) (261 participants), and from a brain bank of the Farmingham Heart Study (FHS) (164 participants). In the UNITE group, this study completed an extensive repeated head injury (RHI) and sports questionnaire to understand the history of all the participants. The next of kin of the FHS group completed an identical athletic history assessment. Participants' exposure to RHI was defined by the total number of years a contact sport was played or years of service in the military. Prior to death,

the UNITE group participants' mood, behavior, cognitive, and clinical statuses were evaluated by online surveys (18).

The neuropathological diagnosis of CTE was made using the National Institute of Neurological Disorders (NINDS) consensus criteria that is rated on a scale of I to IV based on severity, described later in this paper. A diagnosis of Alzheimer's diseases (AD) was made based of the National Institute of Aging Reagan criteria, which included intermediate or high probability. For a participant to be diagnosed with AD, they would have to have sufficient pathology to be classified under the intermediate probability. Lewy bodies were assessed with a-synuclein immunohistochemistry throughout the brain and rated on a scale of 0 to III. These brain regions were viewed by 200x magnification and scored on the following: areas with no Lewy bodies were scored 0, 1-2 Lewy bodies were scored "1," 3-4 Lewy bodies were scored "2," and areas with 5 or more Lewy bodies were scored "3."

Age is a known factor of neurodegenerative pathology and no participants under the age of 50 years old were found to have Lewy bodies or AD. This required comparisons with the CTE pathology groups to be done with participants who had died at an age older than 50 years old. Comparisons were made into four groups based on distributions of the Lewy bodies between the pathological subjects. The groups were LBD, CTE-LBD, AD-LBD, and CTE-AD-LBD. Participants with CTE-LBD had higher deposition of Lewy bodies in the brainstem and lower deposition in the amygdala-predominant compared to AD-LBD. Neocortical deposition of Lewy bodies was 53% higher in CTE-AD-LBD than in the other groups followed by CTE-LBD. Also, Lewy bodies for the CTE-LBD group were found higher in the limbic system compared to the other groups with AD-LBD being the second highest (18).

### Clinical and Exposure Measures Between CTE Pathology Groups

	CTE	CTE-LBD	CTE-AD-LBD	p Value
Sample size (n)	86	36	17	–
Age of First Exposure	12.6 (0.3)	12.2 (0.5)	12.2 (0.9)	0.777
Years of Exposure	16.5 (0.7)	15.9 (1.1)	15.7 (2.3)	0.856
Age at Death	70.1 (1.1)	73.5 (1.6)	77.2 (2.6)	0.020
CTE Symptom Duration	19.1 (1.8)	14.9 (1.7)	23.9 (7.2)	0.169
Symptom Latency	38.7 (1.8)	43.8 (2.3)	35.1 (6.5)	0.226
CTE Stage	3.13 (0.10)	3.00 (0.17)	3.44 (0.30)	0.350
Diffuse A $\beta$ Plaques	47.8%	82.8%	100%	<0.001 <sup>*,†,‡</sup>
Neuritic A $\beta$ Plaques	34.3%	58.6%	100%	<0.001 <sup>*,†,§</sup>
Dementia	61.4%	91.4%	100%	<0.001 <sup>*,†,‡</sup>
Parkinsonism	19.5%	60.0%	30.8%	<0.001 <sup>*,†</sup>
APOE $\epsilon$ 4 presence $\pm$ (%)	28/53 (34.6%)	15/19 (44.1%)	7/8 (46.7%)	0.495 <sup>*</sup>

Numbers are presented as mean (SEM); subjects <50 years-old excluded.

<sup>\*</sup> $\chi^2$  test for proportions.

<sup>†</sup>p < 0.05 for comparison between CTE and CTE-LBD.

<sup>‡</sup>p < 0.05 for comparison between CTE and CTE-AD-LBD.

<sup>§</sup>p < 0.05 for comparison between CTE-LBD and CTE-AD-LBD.

**Table 1: Table showing measurements CTE, CTE-LBD, and CTE-AD-LBD groups comparatively (18).**

Key findings of this study were that individuals known to have CTE had an overall lower average age of death. It also showed a substantial increase in dementia and parkinsonism's within patients when both CTE and LBD were observed compared to CTE alone. Dementia in patients rose 30% from 61.4% (seen in CTE alone) to 91.4% when CTE and LBD were both present. Only 19.5% of patients who were diagnosed with CTE alone had parkinsonism's.

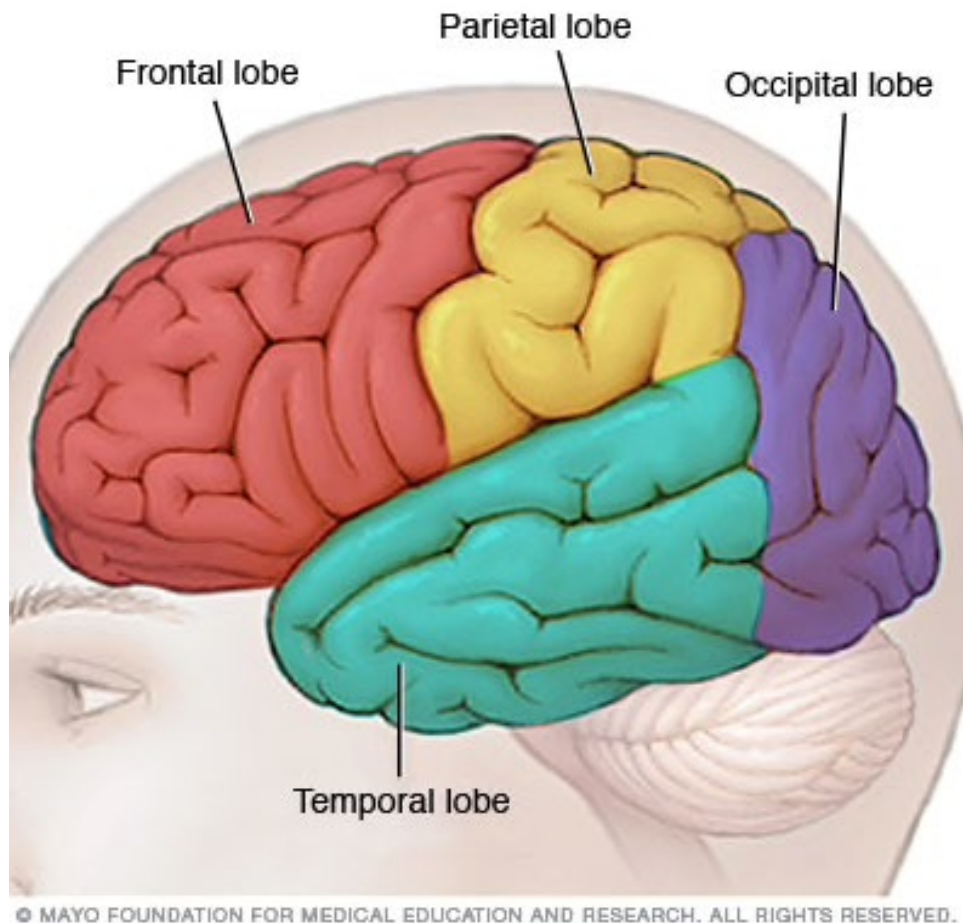
Parkinsonism's in patients who qualified as having both CTE and LBD jumped to 60% (Table 1). Although these pools were not equal, there is an obvious association between Lewy bodies being present and an increase of these symptoms. The study also showed that individuals who had more years of exposure to contact sports were more likely to have neocortical Lewy body accumulation. Those who had 8 or more years of exposure of contact sports were 6.24 times more likely to be diagnosed with LBD compared to those with 7 or less years of exposure (18).

#### *Anatomy:*

Gross anatomical abnormalities have been observed during autopsies in individuals with a pathology currently associated with CTE. These abnormalities are due to neuronal loss caused by phosphorylated tau accumulation. Some of these abnormalities include: reduction of total brain weight, enlargement of ventricles, atrophy of functional brain structures, cavum septum pollicidum, and depigmentation of the locus coeruleus and substantia nigra (2). These abnormalities differ between patients depending on when TBI's occurred. To have proper understanding of these abnormalities and possible symptomatic effects they may have on an individual, one must first know the basic anatomy of the major parts of the brain affected by CTE.

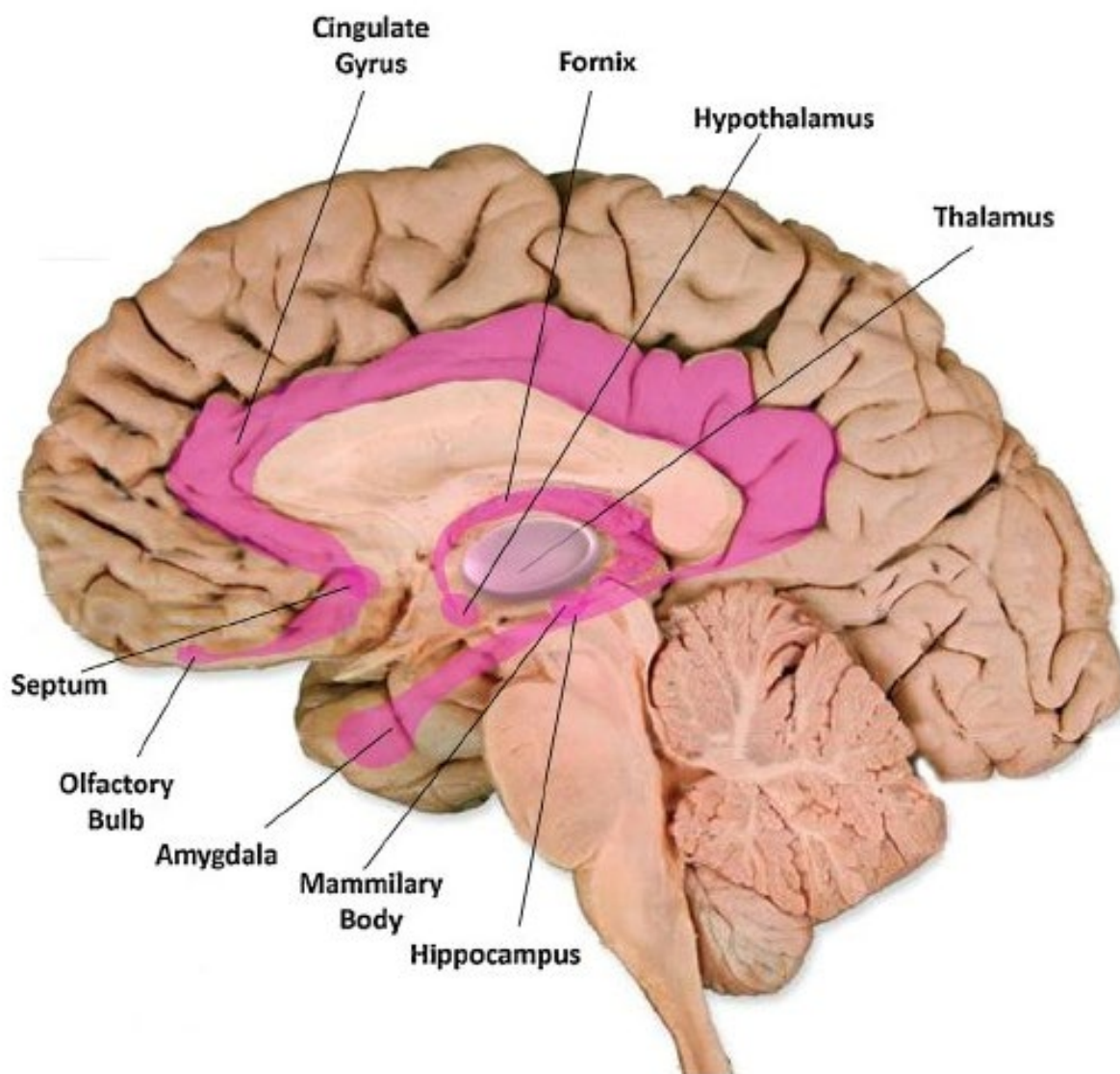
The first part, the cerebrum, consists of the right and left hemisphere, which work together to control the body. Within each hemisphere are four lobes, named regionally: the occipital, temporal, parietal, and frontal lobes. Each lobe hosts specific higher level brain functions. The occipital lobes are responsible for processing vision and the temporal lobes process memories and relate them to senses. The parietal lobes register temperature, touch, and movement, whereas the frontal lobes control movement and cognitive function (Figure 3)

(32, 33).



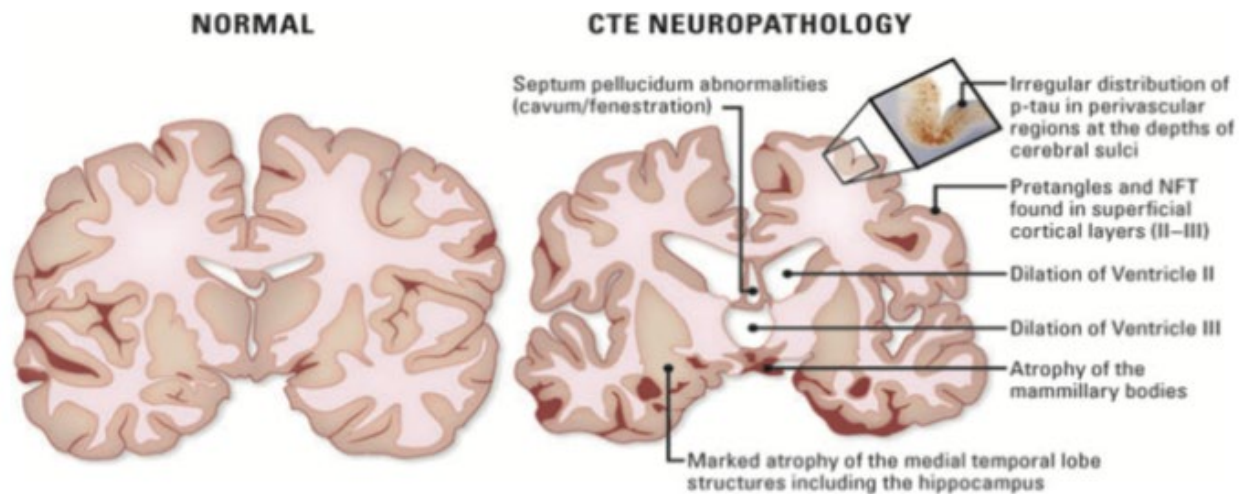
**Figure 3: Regional lobes of the cerebrum and their overlying cortices.** This figure shows the cerebral lobes and cortex regions affected by CTE (32).

A major part of the cerebrum is the cerebral cortex. The cerebral cortex is the outermost surface of the cerebral hemispheres and is known to be the location of where the highest functions are carried out. The cerebral cortex consists of ridges, called gyri, and valleys, called sulci, that add surface area to the cortex, which increases the amount of gray matter for information storage. The cerebral cortex is divided into four regional areas that correspond to the cerebrum; they possess specific functions such as learning, remembering, and language and sensory processing (32, 33).



**Figure 4: The limbic system of the brain.** This figure shows some of the limbic system structures affected by CTE. The mammillary body, hippocampus, amygdala and thalamic region (42).

Another major area of the brain affected by CTE is the limbic system. A few important structures within the limbic system are the amygdala, thalamus, hippocampus, and mammillary bodies. These structures are interconnected and control emotions, mood, and learning, as well as short and long term memory (Figure 4) (43). Damage to these specific areas from TBI's could help explain the various symptoms seen in individuals with CTE.



**Figure 5: This shows general abnormalities caused by CTE.** Macro and microscopic abnormalities between a normal brain (right) and a brain with signs of CTE (right) (46).

### Signs and Symptoms of CTE:

Upwards of 90% of TBI's are classified under what is commonly known as a concussion and the symptoms associated with these concussions are usually resolved in two weeks or less (8). Individuals who have suffered mTBI and whose symptoms last longer than three months may be diagnosed with post concussive syndrome. The symptoms known to be associated with post concussive syndrome are varying in severity by subject, including memory problems, irritability and other personality changes, sensitivity to light and noise, sleep disturbances, depression and other psychological problems, and loss of smell and taste. (9). Multiple brain injuries, or concussions, can lead to a higher prevalence of post-concussive symptoms and into the neuropathological disease we know as CTE.

### *Depression:*

Depression is one of the most common symptoms of CTE and is seen more in individuals who suffer a TBI than in the general population (8). A history of concussion is associated with 3.3-fold greater risk for a diagnosis of depression in adolescents as well as a 2-fold increase in



the elderly (8). Besides single injury risk, the risk for developing depression is greatly increased by the number of concussions sustained by an individual. The relative risk of developing depression increases from 1.5 to 3 when individuals report less than 3 versus 3 or more concussions (9).

Depression treatment responsiveness is inconsistent the majority of the time, and even more so for those who suffer from TBI's (7). A patient having a history of substance abuse, psychosocial factors, and mood disorders may contribute to the risk of developing depression following TBI (8). This is why, without a complete understanding of the pathophysiological mechanism(s) that lead to depression following TBI, targeted disease-modifying therapies cannot be developed (7). There is no individual mechanism currently identified as the cause of depression after TBI, but accumulating evidence has shown that inflammatory pathways may contribute to its development (48). Knowing that inflammation can persist long after TBI, we can then look at the association between the inflammation caused by the TBI and depression following (22). A few studies have shown a possible link between increased pro-inflammatory biomarkers in cerebral spinal fluids (CSF) following a TBI and increased odds of developing depression (43).

#### *Cytokine Regulation:*

Cytokines are a category of signaling proteins that act by binding to specific receptors. Cytokines can be seen acting on cells that release them via autocrine action, cell to cell signaling in local tissue via paracrine action, or long distance signaling via endocrine action. Cytokines are usually produced in a cascade producing other cytokines that can work synergistically or antagonistically (26). Cytokines are usually linked to the immune system where they are

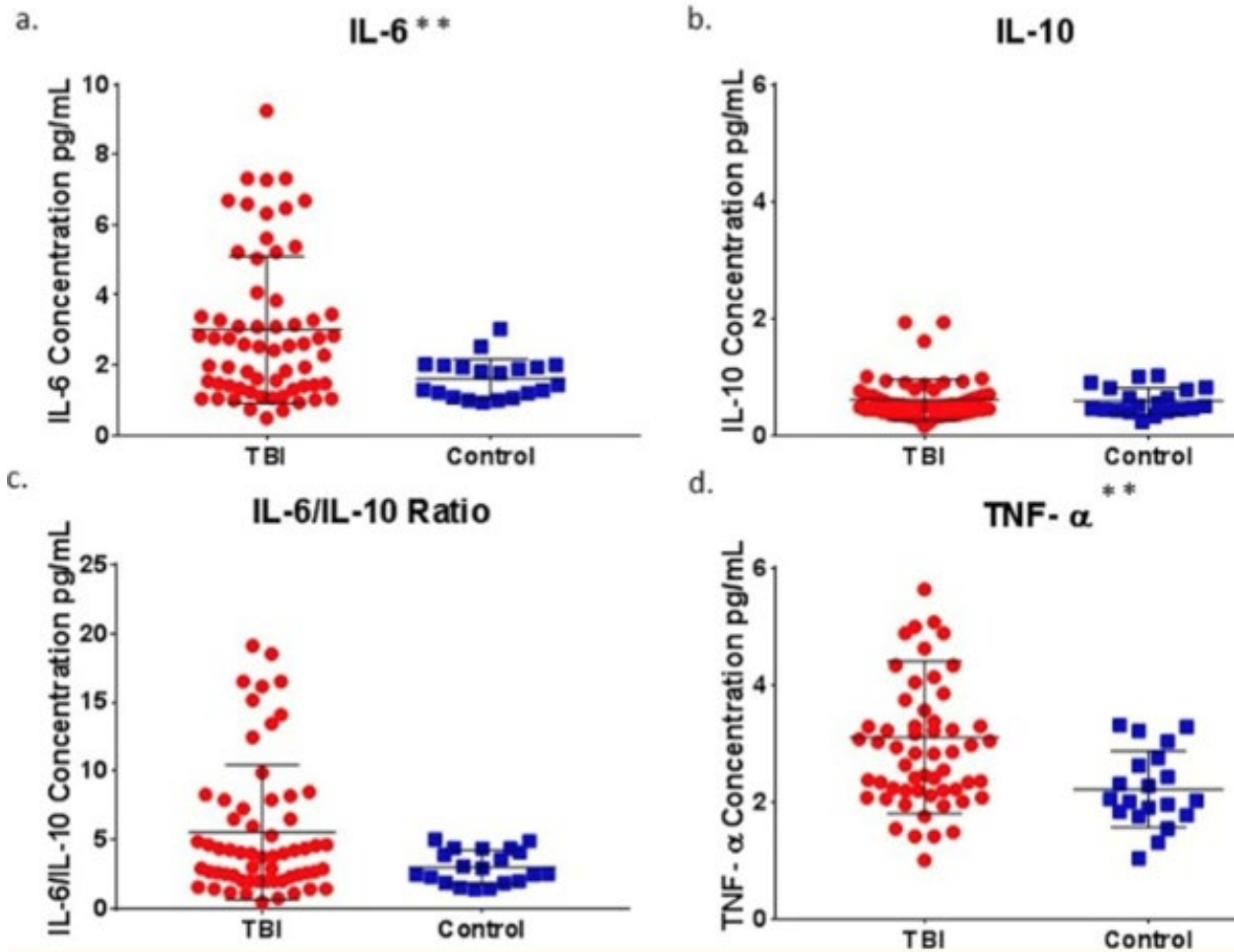


released for defense against pathogens. The cytokines focused on here promote inflammation when released. In a normal body, cytokines are produced in small levels and can increase up to 100 fold more than normal levels when responding to an activating stimulus, such as a pathogen or, in our case, tissue damage.

There are four ways to measure cytokine changes following TBI: in blood, cerebrospinal fluid (CSF), microdialysis, and biopsy of brain tissue. Taking measurements from blood is the least invasive approach by measuring cytokine levels in plasma or serum. In cases of moderate to severe TBI's, CSF that would normally be discarded while attempting to lower the intracranial pressure is saved for biomarker assays. During microdialysis, cerebral probes can be surgically implanted and measure local levels of cytokines (7).

The majority of studies on cytokine levels following TBI's has been done with moderate to severe TBI's, but recently a study was done on military personnel who suffered TBI's showing that an elevation in interleukin IL-6 and tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) in plasma were associated with post-traumatic stress disorder (PTSD) and depression (28). In this study, 83 participants, who were all deployed the same time span 16 months previously, were given a Warrior Administered Retrospective Casualty Assessment Tool (WARCAT) and self-reported PTSD, depression, and quality of life assessments. The WARCAT was used as a self-reporting tool to assess post deployment and war-related TBI's. Out of the 83 participants, 20 who self-reported no TBI's were used as the control and the remaining 63 were used as the TBI group. PTSD was measured using a PTSD military checklist that scores 0-80 with high scores correlating to increased severity.

## Cytokine Concentration in TBI vs. Control



**Figure 5: Differences in plasma cytokine levels.** Significant differences were found in IL-6 and TNF- $\alpha$  (a and d) but not for IL-10 or IL-6/IL-10 Ratios (b and c). TBI, Traumatic Brain Injury; TNF, tumor necrosis factor; IL, interleukin (28).

Scores over 50 were identified as being PTSD positive participants. Depression was measured by using the Quick Inventory of Depressive Symptomatology (QIDS), scoring symptoms from 0 to 7, with higher scores indicating increased severity. Participants with total scores over 13 were considered to have depression (29). More information on the QIDS can be seen in the supplementary materials. WARCAT scores for the TBI participant group averaged 48.7, whereas the non-TBI group averaged a lower 32.5. A similar trend was seen with the QIDS scores as well

with the TBI group having an average score of 12.4 and the non-TBI average score being 7.7 (28). Plasma samples were then taken from the participants, centrifuged frozen at  $-80^{\circ}\text{C}$ , and then thawed and analyzed for cytokines as a single batch, results appear in Figure 5.

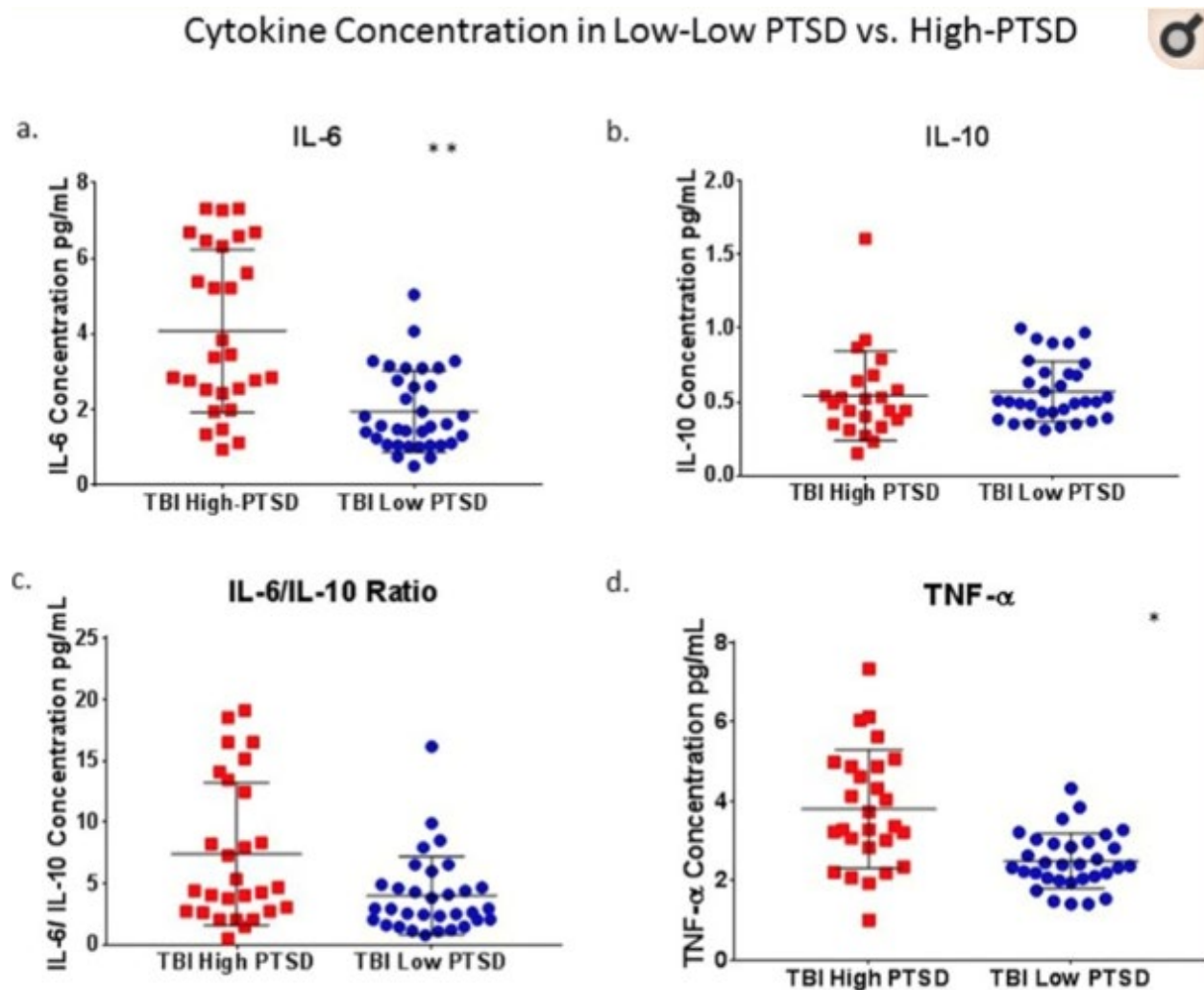
As Figure 5 shows, IL-6 and TNF- $\alpha$  levels are shown to be significantly elevated in participants who suffered TBI versus the non-TBI participants. IL-6 and TNF- $\alpha$  are known pro-inflammatory cytokines and IL-10 is an anti-inflammatory cytokine, as stated earlier the pro-inflammatory cytokines are associated with increased PTSD and depression symptoms.

Below, in Figure 6, are the levels of cytokines in participants who are a part of the TBI group and determined to be either low-PTSD and QIDS scores or high-PTSD and QIDS scores. Even within the TBI participant group, significant differences appear again between IL-6 and TNF- $\alpha$  pro-inflammatory cytokines being most elevated in the high PTSD and QIDS score group.

These results not only show that TBI relates to elevated pro-inflammatory cytokines, but also that high levels of pro-inflammatory cytokines relate with elevated PTSD and comorbid depression (28). Another significant finding of this study is the illustration of elevated cytokine levels months after the occurrence of TBI, which shows that there can be chronic elevation of cytokines and inflammation after TBI.

The evidence supporting the inflammatory theory of depression is correlative and relies of the presence of cytokines elevated in the blood or CSF of depressed individuals compared to healthy controls (7). A meta-analysis of 82 studies showed that peripheral levels of cytokines and chemokines, including IL-6 and TNF- $\alpha$ , were elevated in individuals with major depressive disorders compared to a healthy control (43). Another analysis furthered showed a correlation between cytokine levels and depression in that they found that cytokine levels decreased after

the implementation of anti-depression medication (43).



**Figure 6: Comparison of cytokine levels in military personnel who suffered TBI's and have been diagnosed with low PTSD (N=35) and high PTSD (N=28).** Significant differences were found for IL-6 and TNF-α (a-d) but not for IL-6 or IL-6/IL-10 ratios (b and c) (28).

TBI, Traumatic Brain Injury; TNF, tumor necrosis factor; IL, interleukin.

Also, when interferon cytokines are used as a treatment for hepatitis and multiple sclerosis, there is a strong association with the development of depression, with up to 80% of patients on type 1 interferon therapy developing depression (46). Another link between cytokines and depression is that depression has seemed to be reduced when individuals have been treated with an anti-cytokine treatment compared to control groups. A review and meta-analysis of 16

studies showed a major reduction of depression symptoms compared to a placebo (49).

Cytokines play a major role in Cytokines IL-1 and TNF- $\alpha$  directly play a role in serotonergic signaling (28). They have been shown regulate the serotonin transporter (SERT) by increasing the production of SERT and recruiting it to the membrane (28). SERT transports serotonin from the synapse back into the pre-synaptic neuron (10). Serotonin plays an important role in neuronal circuits, controlling mood and temperament (12). This increase in SERT leads to the decrease of serotonin in the neuronal synapse, which has been shown to increase depression (12).

In the hippocampus, TNF- $\alpha$  has significant effects on glutamate signaling by altering the expression, composition, and phosphorylation of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA), which effect duration of AMPAR-mediated spontaneous excitatory postsynaptic currents (8). Glutamate is an excitatory transmitter in the brain important for memory and learning. TNF- $\alpha$  effects on AMPAR activity appear to be concentration and exposure length dependent. An example of this is during an acute exogenous exposure of TNF $\alpha$  when an immediate increase in AMPAR surface expression and activity have been reported. In contrast, chronic exposure to low levels of TNF $\alpha$ , as in the case of normal aging, decreases AMPAR's (8). TNF- $\alpha$  not only has an affect on excitatory neurotransmitters, but it also affects  $\gamma$ -aminobutyric acid (GABA) receptors. GABA is an inhibitory neurotransmitter in the brain that prevents stimulation by glutamate; this results in a calming sensation in the brain. Dysfunction of GABA receptors can play a role in psychiatric disorders and major depression (22). Specifically, TNF $\alpha$  acting on neurons causes a rapid internalization of GABA<sub>A</sub>R, which decreases its inhibitory synaptic strength (Pribiag and Stellwagen, 2013). The effects

cytokines have on AMPAR and GABA receptors could potentially play a major role in the depressive symptoms following TBI and as seen in CTE.

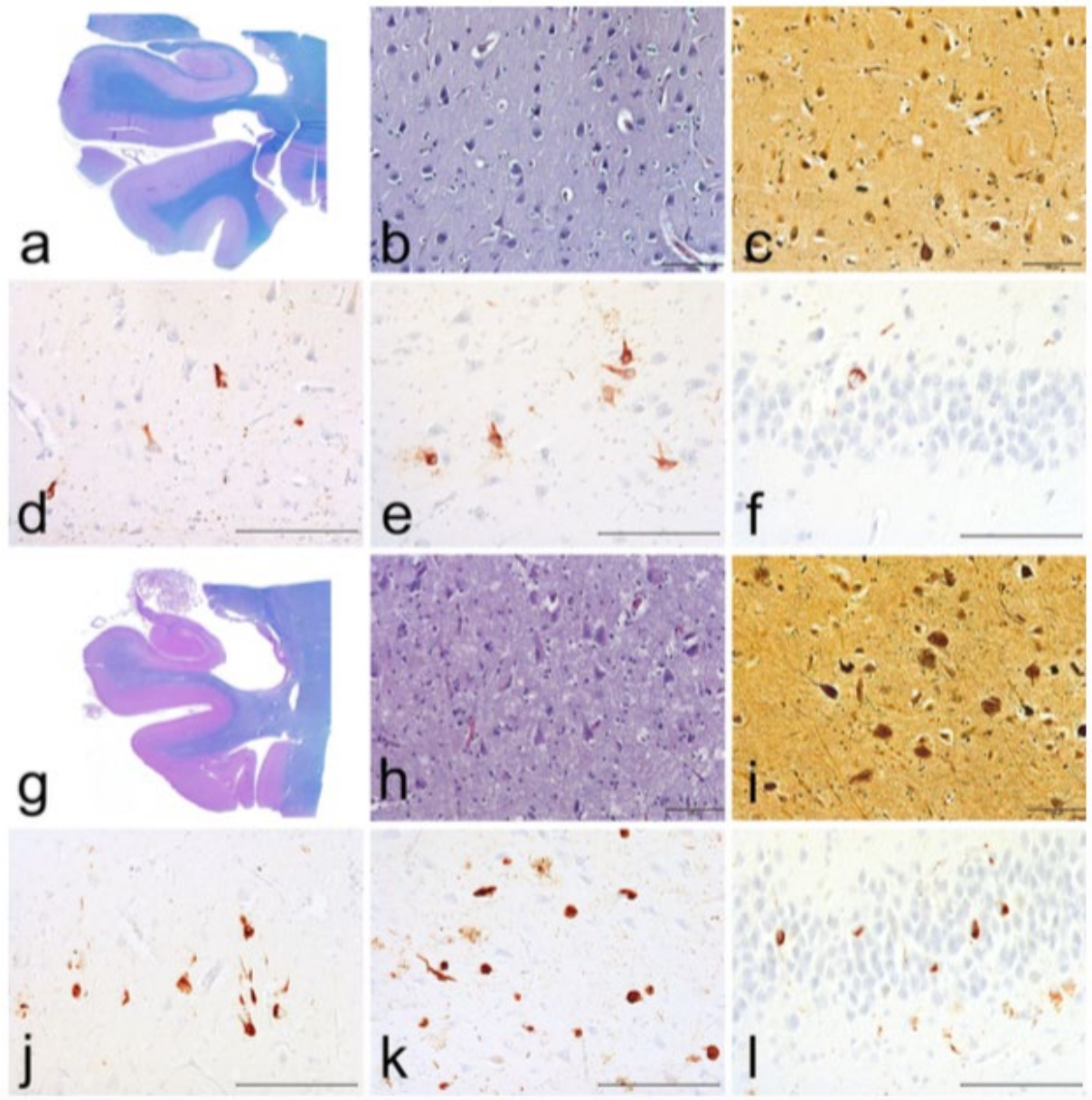
#### *Behavioral Changes:*

Behavioral changes in patients suffering from CTE are hard to assess and usually are reported post mortem by relatives of the patient. There have been many reports of this, but one of the most famous is of American football player Mike Webster. Mike Webster's battle against CTE was made into a movie titled *Concussion*. He was the first individual to be diagnosed with CTE by Dr. Bennett Omalu in 2002 (38, 39). Family members reported impulsive aggression, rapid mood swings, and low interest in normal activities. These behaviors seemed unexplained until after Mike's death in 2002. Now, and since, these behaviors and more have been deemed clinical symptoms of CTE.

Although no definite mechanism has been determined for these behavior changes, a possible explanation could be from the neuronal damage caused P-tau accumulation in mood controlling areas of the brain. These initial symptoms could be due to damage to the limbic system resulting in behavioral symptoms, such as emotional lability, aggression, and violence (13). Other factors could possibly be the accumulation of Lewy bodies in these same areas, as seen in study mentioned above.

#### *Memory Loss:*

Another symptom associated with CTE is memory loss. Temporary memory loss can be seen in patients instantly following TBI, but usually subsides after a few weeks (9). This can be caused by intense inflammation, brain bleeds, or other damages resulting from the TBI. In CTE, memory loss is not instantaneous, as in some TBI's, but slowly progresses over time.



**Figure 7: Comparison of hippocampal pathology in two cases of moderate CTE.** In example 1 (a–f), there is **a** mild hippocampal atrophy, **b** mild neuronal loss in CA1, **c** sparse NFTs in CA1 **d** sparse NFTs in CA1, AT8 immunostain, **e** moderate numbers of diffusely immunopositive AT8 stained neurons in CA4, and **f** occasional AT8 immunopositive NFTs in the dentate gyrus. In example 2 (g–l), there is **g** more severe hippocampal atrophy, **h** clear neuronal loss in CA1, **i** moderate density of NFTs in CA1, Bielschowsky silver stain, **j** moderate density of NFTs in CA1, AT8 immunostain, **k** high numbers of AT8-stained neurons and NFTs in CA4, and **l** moderate numbers of AT8 immunopositive NFTs in the dentate gyrus (40). Bars indicate 100  $\mu$ m.

This could possibly be explained by the mechanism of injury and how p-tau propagates through brain tissues. Location of beginning p-tau formation may be a significant aspect of the slow onset of symptoms presented by a patient. P-tau in stage one CTE patients is seen accumulated on the outer cortex of the brain (McKee). As stated above, excessive accumulation of p-tau causes neuronal dysfunction and death. Knowing that regions of the cerebral cortex are used for memory and learning, P-tau damage to these areas can cause memory loss. As time progresses, the P-tau migrates into deeper regions of the brain, causing atrophy to important memory related structures such as the hippocampus and amygdala. This is why more severe memory loss can be seen in patients that progress to a higher stage of diagnoses (Figure 7).

As stated in the previous section, glutamate is an important neurotransmitter associated with memory and learning. Additionally, the number of its receptors, AMPAR, can be reduced by TNF- $\alpha$  when expressed at chronic low levels (22). As seen in TBI's, there is a chronic elevation of cytokines such as TNF-  $\alpha$  above basal levels. Potentially, a long term elevation of TNF- $\alpha$  could be one reason why individuals with CTE have problems with memory and learning. Further, with CTE being a comorbid disease, other diseases such as Lewy body disease and Alzheimer's disease could play a role in the degradation of memory. As seen above, Lewy Bodies are a possible source of the dementia symptoms demonstrated by CTE-LBD patients.

### **Diagnosis:**

Diagnosis of CTE so far has only been done post mortem in patients. The standard scale for the diagnosis of CTE was proposed in 2013 and staged CTE I through IV, with IV being most severe. In 2013, the neuro-pathological diagnosis of CTE were proposed, as follows:

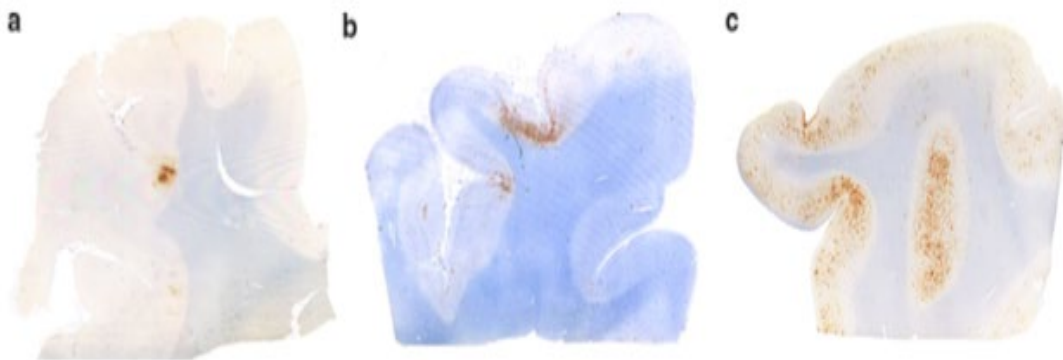
1. Perivascular foci of p-tau immunoreactive NFTs and ATs in the neocortex



2. Irregular distribution of p-tau immunoreactive NFTs and ATs at the depths of cerebral sulci
3. NFTs in the cerebral cortex located preferentially in the superficial layers (often most pronounced in temporal cortex)
4. Supportive, non-diagnostic features: Clusters of sub-pial ATs in the cerebral cortex, most pronounced at the sulcal depths. (3)

Abbreviations: NFTs =Neurofibrillary tangles, ATs = astrocytic Tangles

A study performed in March 2013 determined that the diagnostic neuropathological lesion of Chronic Traumatic Encephalopathy (CTE) was different from other taupathies. The pathognomonic lesion of CTE consists of p-tau aggregates in neurons, astrocytes, and cell processes around small vessels in an irregular pattern at the depths of the cortical sulci (Figure 8) (3).



**Figure 8: Low magnification of phosphorylated tau (p-tau) stained slides showing the spatial pattern in CTE pathology in cortical sulci.** Three different cerebral cortex slides from 3 separate CTE cases. The darker brown regions are areas of stained p-tau proteins (3).

It was also determined that the p-tau was detectable at a lower power than other

tauopathies and in an irregular pattern (3). This pattern consisted of astrocytic tangles, dot and thread like neurites located in the depths of sulci and along penetrating blood vessels. This study also discovered that CTE wasn't a single diagnosis, but is considered a co-morbid disease. Staging diagnosis for CTE was based of work from Braak and Braak who determined that there are 6 different stages based on characteristic distribution of NFT in patients who have AD. This staging system is the base of diagnosing AD used by the Nation Institute on Aging (3). The diagnostic stages of CTE are:

**I:** Brains that are in stage I show no gross anatomic or macroscopic differences compared to normal brains. Microscopically, they show small isolated perivascular focal epicenters of p-tau NFTs and neuropil neurites. These NFTs and neuropil neurites are characterized as dot and thread like. Tau pathology is most commonly localized to the depths of the cerebral sulci of the frontal, temporal, insular, septal and parietal cortices, although there may also be sparse NFT scattered throughout the adjacent cortex.

**II:** Subtle macroscopic changes within the brain may occur in Stage II. This could be mild enlargement of the frontal horns of the lateral ventricles and third ventricle; cavum septum pellucidum and the locus coeruleus and substantia nigra may also become pale. Multiple epicenters of p-tau will be found in the cortices and AT are seen in sub-pial regions. In cortexes adjacent to p-tau epicenters, NFT are seen throughout cerebral layers.

**III:** The majority of brains in stage III have macroscopic changes but little anatomical change. Usually, there is a reduction in brain weight, mild atrophy of the temporal and frontal lobes, and enlargement of the lateral and third ventricles. Also, atrophy is seen in

the mammillary bodies, thalamus, and hypothalamus, as well as the corpus callosum becoming thin. Half of subjects have septal abnormalities. The cavum septum pellucidum and locus coeruleus become pale. Microscopically, patches of merging NFT, NT's, and AT are found around blood vessels in sulcal depths and in superficial laminae of the cortex. NFT can be seen in the olfactory bulb, hippocampus, entorhinal cortex, amygdala, hypothalamus, mammillary bodies, nucleus basalis of Meynert, substantia nigra, dorsal and median raphe nuclei, and locus coeruleus. Neurofibrillary degeneration of the hippocampus can be seen.

**IV:** Stage IV CTE brains show substantial decrease in brain weight with pronounced atrophy of cerebrum, frontal and temporal lobes, medial temporal lobes, mammillary bodies, and thalamus. Wide spread atrophy of white matter can be seen. Thinning of corpus callosum and hypothalamic floor are common. Septum abnormalities can be seen, including cavum septum, perforations, fenestrations or total absence of the posterior septum. The cavum septum pellucidum and locus coeruleus are pale.

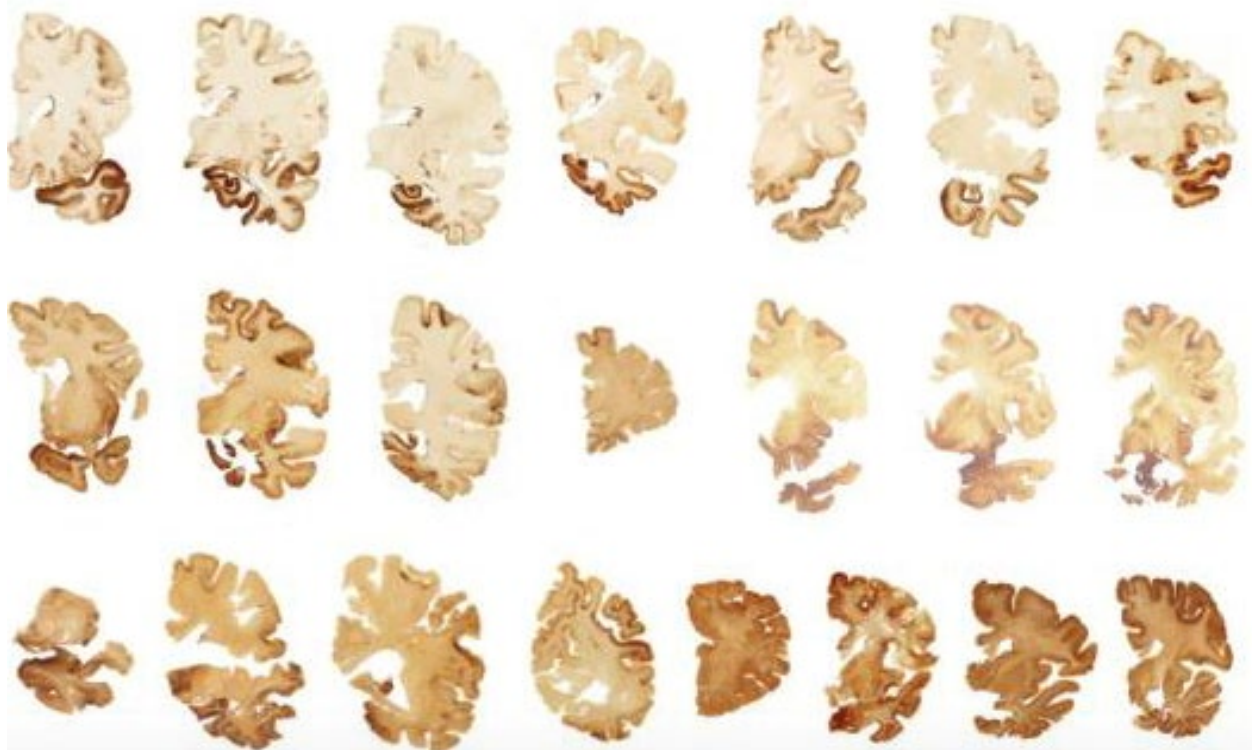
Microscopically, diffuse myelin loss, astrocytosis of the white matter, and neuronal loss in the cerebral cortex, hippocampus and substantia nigra occur. P-tau pathology is densely distributed throughout the cerebrum, thalamus, hypothalamus, mammillary bodies, basal ganglia, brainstem, cerebellar dentate nucleus, and, occasionally, spinal cord. The visual cortex is usually a spared area in this disease. NFT, most of which are extracellular, are found throughout the hippocampal formation, including the dentate gyrus. Severe neuronal occurs loss in the hippocampus. There is marked loss of myelinated nerve fibers, axonal dystrophy and loss throughout the cerebral and

cerebellar white matter.

Stage one (I) CTE



Stage Four (IV) CTE



**Figure 9: Comparison of stage I CTE to stage IV CTE.** Stage I shows small p-tau (brown spots) forming in the cortical sulci. Stage IV illustrates widespread severe p-tau pathology, affecting most regions of the cerebral cortex and the medial temporal lobe but sparing the visual cortex in all but the most severe cases (40).

**Treatment:**

With diagnosis of CTE being done post mortem, there is no definitive treatment at this time. Preventative measures while participating in contact sports or military related events can help avoid TBI's. Wearing the correct protective equipment and avoiding unnecessary collisions or concussive blasts is the best way to avoid TBI's. Also, athletes and military personnel should follow their organizations' concussion protocols after experiencing a TBI to prevent further damage. Treatment of symptoms of CTE may be done to help improve quality of life of the patient. Studies have considered lowering depression symptoms by attempting to lower pro-inflammatory cytokines in patients following a TBI. Blocking these signals could prevent inflammation and excitatory damage to neurons caused by the dysregulation of the glutamate and GABA signaling (7). Treatments with cytokine inhibitors has been shown to promote functional recovery in learning and memory tasks in TBI mouse models when implemented one week after sustaining the injury (35). Experiments have also shown that, following a TBI, neuro stem cells (NSC) may help in a regenerative process of the brain (35). This has sparked the interest of researchers to see if stem cell implementation could possibly be used as treatment method. Two methods of stem cell treatment are currently being investigated. The first is the modulation of naturally occurring endogenous NSC reservoirs within the patient and the second is implanting exogenous NSC into the patient to assist regeneration (35). Both methods have shown positive results in treatment of TBI's in animal models but the human neurogenesis process is still unclear. A potential issue with this treatment process is that areas where a TBI

occurred may be too severely damaged and too hostile for the transplanted NSC to survive (35). Further, generation of sufficient functional NSC to be implanted has been proven difficult to do. Treatments adding Omega-3 fatty acids to a person's diet has also shown to help combat TBI symptoms (50). Omega-3 fatty acids have been shown to help with neuro-synaptic transmission and improving neuroplasticity. Other possible treatments like behavioral therapy and memory exercises can also help deal with symptomatic mood swings and difficulty with recall.

### **Conclusion:**

#### *Limitations:*

With the brain being encased in the skull, it is difficult to study without its removal. This is the most significant limitation encountered while researching CTE; it limits the number of participants available for studies. Additionally, with CTE only being heavily researched in recent years, little information of the living history of the patients used in most of these studies exists. Knowledge of the p-tau mechanism also remains incomplete, which makes it difficult when trying to create a treatment to inhibit its toxicity. The majority of the studies that have shown cytokine changes in individuals following TBI have been done in patients who have suffered a moderate to severe TBI. There has been limited data from individuals who suffer from mild TBI. Further, few studies have been done longitudinally for cytokine biomarkers within the blood or CSF. Knowing how long cytokines last and when possible increase may occur in the body could help prevent chronic inflammation in the brain.

#### *Future Research:*

Future research in this field could occur in a wide range of areas. The most demanding areas of concern would be diagnosis of CTE in patients who are still living. A current study,

started in 2014, named Understanding Neurological Injuries and Traumatic Encephalopathy (UNITE), is working towards this. Their goal is to find associations in living patients at risk of CTE that can be then confirmed postmortem. Their hope is that this information could be used for further clinical diagnoses of CTE. Also, there are studies trying to solve this issue by tagging the p-tau protein with a marker that can be seen by Positron emissions testing (PET) scans. These studies hope that early diagnoses of the disease can be achieved through observing the accumulation and binding patterns of these P-tau proteins. Currently, the mechanism of neurotoxic p-tau is little understood. Further research needs to be done in order to understand the full pathogenesis of the toxic proteins and ways to prevent its propagation in CTE. Another future field of experiments is looking at biomarkers such as elevated levels of P-tau and cytokines in CSF. This information could also be used to help in early diagnosis. Over the past decade, a lot of information and associations have been discovered involving this disease, but funding and future discoveries are needed in hopes of finding a cure.

## References and Notes:

1. American Association of Neurological Surgeons, "Symptoms" in *Concussion* (American Association of Neurological Surgeons, Illinois, 2019).
2. McKee, A. C., Cairns, N. J., Dickson, D. W., Folkerth, R. D., Keene, C. D., Litvan, I., Perl, D. P., Stein, T. D., Vonsattel, J. P., Stewart, W., Tripodis, Y., Crary, J. F., Bieniek, K. F., Dams-O'Connor, K., Alvarez, V. E., Gordon, W. A., TBI/CTE group. (2015). The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. *Acta neuropathologica*, 131 (1), 75-86.
3. Martland HK, Kappelmann N, Lewis G, Dantzer R, Jones PB, Khandaker GM (2018). Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol Psychiatry* 23:335-343.
4. Pribyl H, Stellwagen D (2013). TNF-alpha downregulates inhibitory neurotransmission through protein phosphatase 1-dependent trafficking of GABA(A) receptors. *J Neurosci* 33:15879-15893.
5. Hussey HH. Punch Drunk. *JAMA*. 1976; 236 (5): 485.  
doi:10.1001/jama.1976.03270050041032
6. Millspaugh J (1937). Dementia pugilistica. *US Naval Med Bull* 35:297Y303.
7. Bachstetter, A., Bodnar, C., & Morganti, J. (2018). Depression following a traumatic brain injury: Uncovering cytokine dysregulation as a pathogenic mechanism. *Neural Regeneration Research*, 13 (10), 1693. doi:10.4103/1673-5374.238604
8. Blennow K, Brody DL, Kochanek PM, Levin H, McKee A, Ribbers GM, Yaffe K, Zetterberg H (2016). Traumatic brain injuries. *Nat Rev Dis Primers* 2: 16084.
9. Guskiewicz KM, Marshall SW, Bailes J, McCrea M, Harding HP Jr, Matthews A, Mihalik JR, Cantu RC (2007). Recurrent concussion and risk of depression in retired professional football players. *Med Sci Sports Exerc* 39: 903-909.
10. Serotonin Transporter (n.d.). Retrieved March 13, 2019 from  
<https://www.sciencedirect.com/topics/neuroscience/serotonin-transporter>
11. Houwing, D. J., Buwalda, B., van der Zee, E. A., de Boer, S. F., & Olivier, J. (2017). The Serotonin Transporter and Early Life Stress: Translational Perspectives. *Frontiers in*



- cellular neuroscience*, 11, 117. doi:10.3389/fncel.2017.00117
12. Galgano, M. A., Cantu, R., & Chin, L. S. (2016). Chronic Traumatic Encephalopathy: The Impact on Athletes. *Cureus*. doi:10.7759/cureus.532
  13. Critchley M (1949) e. Punch-drunk syndromes: The chronic traumatic encephalopathy of boxers, Maloine, Paris.
  14. Jackson, H. (1986). Macdonald Critchley. *Archives of Neurology*, 43 (5), 435. doi:10.1001/archneur.1986.00520050015013
  15. Critchley, M. (1957). Medical aspects of boxing, particularly from a neurological standpoint. Retrieved from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1974382/>
  16. Brenner, Lisa A., et al. "Growing Literature but Limited Evidence: A Systematic Review Regarding Prebiotic and Probiotic Interventions for Those with Traumatic Brain Injury and/or Posttraumatic Stress Disorder." *Brain Behavior and Immunity* 65 (2017): 57-67. Print.
  17. Mietelska-Porowska, U. Wasik, M. Goras, A. Filipek, G. Niewiadomska Tau protein modifications and interactions: their role in function and dysfunction. *Int. J. Mol. Sci.*, 15 (2014), 4671-4713.
  18. Adams, J. W., Alvarez, V. E., Mez, J., Huber, B. R., Tripodis, Y., Xia, W., ... Stein, T. D. (2018). Lewy Body Pathology and Chronic Traumatic Encephalopathy Associated With Contact Sports. *Journal of neuropathology and experimental neurology*, 77 (9): 757–768. doi:10.1093/jnen/nly065
  19. tauopathy. (n.d.) *Medical Dictionary*. (2009). Retrieved December 5, 2018 from <https://medical-dictionary.thefreedictionary.com/tauopathy>
  20. tau. (n.d.) *Medical Dictionary*. (2009). Retrieved December 5 2018 from <https://medical-dictionary.thefreedictionary.com/tauopathy>
  21. Cryan, J. F., & Slattery, D. A. (2010). GABAB Receptors and Depression: Current Status. *GABAB Receptor Pharmacology - A Tribute to Norman Bowery Advances in Pharmacology*, 427-451. doi:10.1016/s1054-3589(10)58016-5

22. Josephs, K. A. (2015). Tauopathies. *Movement Disorders*, 815-828. doi:10.1016/b978-0-12-405195-9.00053-6
23. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, Gentleman S, Heckemann RA, Gunanayagam K, Gelosa G, Sharp DJ. (2011). Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol* 70: 374-383.
24. Simpson, K. (1981). Forensic Medicine. *The American Journal of Forensic Medicine and Pathology*, 2 (3), 282. doi:10.1097/00000433-198109000-00023
25. The Science of CTE. (2018, November 06). Retrieved November 29, 2018 from <https://concussionfoundation.org/CTE-resources/science-of-CTE>
26. Zhang, J. M., & An, J. (2007). Cytokines, inflammation, and pain. *International anesthesiology clinics*, 45 (2), 27–37. doi:10.1097/AIA.0b013e318034194e
27. Ramamoorthy S, Ramamoorthy JD, Prasad PD, Bhat GK, Mahesh VB, Leibach FH, Ganapathy V. (1995). Regulation of the human serotonin transporter by interleukin-1 beta. *Biochem Biophys Res Commun*, 216: 560-567.
28. Devoto, C., Arcurio, L., Fetta, J., Ley, M., Rodney, T., Kanefsky, R., & Gill, J. (2017). Inflammation Relates to Chronic Behavioral and Neurological Symptoms in Military Personnel with Traumatic Brain Injuries. *Cell transplantation*, 26 (7), 1169–1177. doi:10.1177/0963689717714098
29. Reilly, P., Winston, C. N., Baron, K. R., Trejo, M., Rockenstein, E. M., Akers, J. C. Yuan, S. H. (2017). Novel human neuronal tau model exhibiting neurofibrillary tangles and transcellular propagation. *Neurobiology of Disease*, 106, 222-234. doi:10.1016/j.nbd.2017.06.005
30. Clavaguera F, Tolnay M, Goedert M. Prion-like properties of assembled tau protein. In: Jucker M, Christen Y, editors. *Proteopathic Seeds and Neurodegenerative Diseases*. Springer; Heidelberg, New York, Dordrecht, London: 2013. pp. 87–95.
31. Guo, J. L., & Lee, V. M. (2011). Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. *The Journal of biological chemistry*, 286 (17), 15317–15331. doi:10.1074/jbc.M110.209296
32. Mayo Clinic Brain lobes. (n.d.). Retrieved from <https://www.mayoclinic.org/brain-lobes/img-20008887>
33. Crossman AR, Neary D. *Neuroanatomy*. 2nd. London: Churchill Livingstone; 2000.

34. Webster SJ, Van Eldik LJ, Watterson DM, Bachstetter AD. (2015). Closed head injury in an age-related Alzheimer mouse model leads to an altered neuroinflammatory response and persistent cognitive impairment. *J Neurosci* 35: 6554-6569.
35. Rolfe, A., & Sun, D. (2015). Stem Cell Therapy in Brain Trauma: Implications for Repair and Regeneration of Injured Brain in Experimental TBI Models. *Brain Neurotrauma Frontiers in Neuroengineering Series*, 587-596. doi:10.1201/b18126-49
36. Omalu BI, DeKosky ST, Minster RL, Kamboh MI, Hamilton RL, Wecht CH. Chronic traumatic encephalopathy in a National Football League player. *Neurosurgery*. (2005). 57: 128–134.
37. Omalu BI, DeKosky ST, Hamilton RL, Minster RL, Kamboh MI, Shakir AM, Wecht CH. Chronic traumatic encephalopathy in a national football league player: part II. *Neurosurgery*. (2006) 59: 1086–1093.
38. National Institute of Aging, What Is Lewy Body Dementia? (n.d.). Retrieved from <https://www.nia.nih.gov/health/what-lewy-body-dementia>
39. McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, et al. The spectrum of disease in chronic traumatic encephalopathy. *Brain*. (2013). 136 (Pt 1): 43–64.
40. The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy Ann C. McKee, Nigel J. Cairns, Dennis W. Dickson, Rebecca D. Folkerth, C. Dirk Keene, Irene Litvan, Daniel P. Perl, Thor D. Stein, Jean-Paul Vonsattel, William Stewart, Yorghos Tripodis, John F. Crary, Kevin F. Bieniek, Kristen Dams-O'Connor, Victor E. Alvarez, Wayne A. Gordon, and the TBI/CTE group.
41. <https://concussionfoundation.org/CTE-resources/science-of-CTE>
42. Adjamian, P., Hall, D.A., Palmer, A. R., Allan, T. W., & Langers, D. R. (2014). Neuroanatomical abnormalities in chronic tinnitus in the human brain. *Neuroscience & Biobehavioral Reviews*, 45, 119-133. doi:10.1016/j.neubiorev.2014.05.013
43. Kohler CA, Freitas TH, Maes M, de Andrade NQ, Liu CS, Fernandes BS, Stubbs B, Solmi M, Veronese N, Herrmann N, Raison CL, Miller BJ, Lancot KL, Carvalho AF. Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr Scand*. 2017; 135:373–387.
44. Juengst SB, Kumar RG, Failla MD, Goyal A, Wagner AK. Acute inflammatory biomarker profiles predict depression risk following moderate to severe traumatic brain injury. *J Head Trauma Rehabil*. 2015; 30:207–218.

45. Gilroy A.M. *Anatomy: An Essential Textbook*. (Thieme Medical Publishers, New York, 2013), pp. 397-406.
46. Aldag, M., Armstrong, R.C., Bandak, F., Bellgowan, P.S., Bentley, T., Biggerstaff, S., Zheng, J. (2017). The Biological Basis of Chronic Traumatic Encephalopathy following Blast Injury: A Literature Review. *Journal of Neurotrauma*, 34(S1). doi:10.1089/neu.2017.5218
47. Raison CL, Demetrashvili M, Capuron L, Miller AH. Neuropsychiatric adverse effects of interferon-alpha: recognition and management. *CNS Drugs*. 2005; 19:105–123.
48. Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat Rev Immunol*. 2016; 16:22–34.
49. Kappelmann N, Lewis G, Dantzer R, Jones PB, Khandaker GM. Antidepressant activity of anti-cytokine treatment: a systematic review and meta-analysis of clinical trials of chronic inflammatory conditions. *Mol Psychiatry*. 2018; 23:335–343.
50. Lewis, M. D. (2016). Concussions, Traumatic Brain Injury, and the Innovative Use of Omega-3s. *Journal of the American College of Nutrition*, 35(5), 469-475. doi:10.1080/07315724.2016.1150796

### **Acknowledgments:**

I would like to acknowledge and thank Dr. Elizabeth Droke for her help in reviewing this paper and for her suggestions regarding its research and direction. I would also like to thank my Master's program cohort for their help in proofreading and support during this process.