

**Biomarkers Associated with Longitudinal Cognitive Decline in Veterans with
Traumatic Brain Injury**

Ambika Menon

Department of Biology

Duke University

*Honors thesis submitted in partial fulfillment of the requirements for graduation with
Distinction in Biology in Trinity College of Duke University*

May, 2018

Abstract

Traumatic brain injury (TBI) represents an important medical and public-health problem. One cohort particularly affected by TBI are veterans that have returned from the Afghanistan and Iraq wars. The ramifications of TBIs are multifold, with some of the most common known to include neurodegeneration. Blood biomarkers may provide a minimally invasive diagnostic tool to predict accelerated longitudinal neurocognitive decline. Thirty-one veterans were therefore enrolled in a longitudinal study, with their baseline blood assays and neurocognitive status collected between 2005 – 2007. The blood biomarkers tested at baseline included TNF- α , IL1- β , IL-6, IL-2, pregnenolone, allopregnanolone, progesterone, and APOE isoform status. Two neuropsychological measures of visual attention and a measure of delayed memory were assessed longitudinally in 10 veterans. Pregnenolone and IL-2 levels were found to be lower in veterans with TBI compared with controls. The triple interaction between APOE status, TBI status, and pregnenolone levels was borderline significant, indicating that those with the $\epsilon 4$ isoform will have worse outcomes. While all three measures of cognitive decline were greater in TBI subjects, the attentional measures (Stroop interference and Symbol Search) were statistically significant. All blood biomarkers were negatively related to cognitive decline, as expected, although results were not significant, likely due to the small sample size. Results show promise in the use of blood biomarkers as an effective method of predicting cognitive decline based on TBI status. Thus, further work with a larger sample size is warranted, as the blood biomarker levels may predict neuroplasticity changes causing cognitive decline in those with TBI.

Introduction

Traumatic brain injury (TBI) has become pervasive in veterans due to the wars in Afghanistan and Iraq. Over 2.5 million military personnel have been deployed since 2001 to Afghanistan, Iraq, and regional staging areas for the wars. With the advance in torso protective gear and emergency medicine in theatre, many injuries that would have resulted in death have yielded an increased frequency of head and neck injuries relative to previous wars (Capehart & Bass, 2011). One of the most common forms of head and neck injuries is manifested in TBI, ranging from mild to severe. According to some U.S. Army medical officials, the incidence of TBIs in the post-9/11 active-duty and veteran population is as high as 19% (Tanielian et al., 2008).

Although TBI in its milder forms can vary considerably in the character and persistence of symptom sequelae, moderate to severe TBI typically results in neurocognitive impairment in the domains of attention, memory, processing speed, speech-language, visuospatial abilities, and motor functioning, as well as producing personality changes that may include irritability, impulsivity, anxiety, and depressive affect (Lundin, de Boussard, Edman, & Borg, 2006; Schneiderman, Braver, & Kang, 2008). A post-concussion syndrome can also follow, including headaches, balance disturbances, photosensitivity, changes in sleep and appetite, fatigue, and other symptoms (Lundin et al., 2006; Schneiderman et al., 2008). Importantly, an increased incidence of Alzheimer's disease (AD), chronic traumatic encephalopathy (CTE), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and other similar neurodegenerative disorders has been reported following a history of TBI (Fleminger, Oliver, Lovestone, Rabe-Hesketh, & Giora, 2003; Gavett, Stern, Cantu, Nowinski, & McKee, 2010; Goldman et al., 2006; Sivanandam & Thakur, 2012). Epidemiological studies have found that 30% of those who die

from TBI have a significant number of amyloid fibril plaques, a hallmark of AD (Sivanandam & Thakur, 2012). Amyloid fibrils are formed from the amyloid beta (A β) peptide, which can exist in multiple isoforms. The 40-residue (A β 40) is the most prevalent A β isoform in the brain, while an increase in the 42-residue (A β 42) has been found to be significantly related to certain forms of AD (Schmidt et al., 2009). Similarly, repeated head injury has been found to cause neurofibrillary tangles, which are also present in CTE and PD (Gavett et al., 2010).

Many of the TBIs sustained in Afghanistan and Iraq have derived from the same origins as in previous theatres of war: gunshot wounds, hand-to-hand combat, motor-vehicle accidents, falls, and collisions. However, a novel feature of these wars has been the escalation in the occurrence of blast injury, particularly from improvised explosive devices (IEDs; Capehart & Bass, 2011). Between 2001 and 2005, IEDs were responsible for over 80% of all military casualties in these wars (Bird & Fairweather, 2007). In blast-induced neurotrauma (BINT), the most common variety of TBI, four manifestations of injury can be identified: primary effects caused by the incident overpressure from the blast wave and subsequent blast wind, secondary effects from shrapnel and other propelled objects, tertiary effects caused by bodily displacement into stationary objects, and quaternary effects involving burns, asphyxia, or toxic chemicals (Bochicchio et al., 2008; Bumbaširevic, Lesic, Mitkovic, & Bumbaširevic, 2006; DePalma, Burris, Champion, & Hodgson, 2005). Blast injuries have accounted for 63% of all TBI diagnoses in the Afghanistan and Iraq wars (Bass et al., 2012). Most TBIs involve closed-head injury. Penetrating injury is far less common, but when it occurs, it is highly revealing of the injury and the accompanying diagnosis, as well as introducing additional complications that may include infection (Martin, Lu, Helmick, French, & Warden, 2008). Mild closed-head injury, however, is much more difficult to diagnose in the absence of medical observation. Due to its

variety of clinical presentations, blast often results in a 36% misdiagnosis rate (Bochicchio et al., 2008). Therefore, improved diagnostic tools are imperative to properly address this nosological challenge.

A long latency between the occurrence of a TBI and the neurodegenerative disorders of later life is typical (Goldman et al., 2006; Langlois et al., 2003). This phenomenon arises from the common occurrence of TBI in young age, especially for military personnel, yet the presentation of symptomatology in middle-aged or elderly persons unfolds over many years. A means of discriminating the prognostic likelihood of accelerated cognitive decline following from TBI would thus be of great value in prophylaxis, early initiation of treatment, and care planning. One approach that may assist in diagnosis is an examination of blood biomarkers with maximal promise in predicting neurodegenerative disease mediated or moderated by TBI. Specification of the neurobiological pathways leading from neuronal injury to initiation of neurodegenerative disease processes and their long-term expression would significantly enhance the development of pharmacotherapies, intracranial stimulation and implantation approaches, and other therapeutic modalities.

This introduction thus reviews prior literature describing blood biomarkers with the greatest promise of predicting the transition of TBI into neurodegenerative disease. Neurocognitive testing and phlebotomy represent the least invasive and most economical techniques for assisting diagnosis and prognosis, thus potentially benefitting the largest number of patients. The present study thus addresses this diagnostic challenge by examining candidate longitudinal biomarkers of TBI which may predict cognitive decline. We examine two major types of biomarkers: 1) peripheral blood proteins, including TNF- α , IL1- β , IL-6, IL-2, pregnenolone, allopregnanolone, and progesterone and 2) the gene most strongly associated with

the development of AD, apolipoprotein E (APOE). We examine cognitive decline through three specific neurocognitive test measures: Stroop Color-Word Test, California Verbal Learning Test-II (CVLT-II) delayed free recall, and Wechsler Adult Intelligence Scale-Third Edition (WAIS-III) Symbol Search. We provide a model of TBI effects over time and examine biomarkers that are associated with separate phases of brain injury and recovery and how they may relate to cognition changes over 10 years.

Model of Longitudinal TBI Neurobiology

Mechanisms of injury to the brain in TBI can entail three fundamental varieties: direct destruction of neurons such as occurs with penetrating injuries or fragmentation of the skull, rapid acceleration followed by deceleration due to translational forces, and rapid acceleration-deceleration due to angular momentum. Upon impact to the skull, a coup injury can occur at the site of impact. The brain has a gelatinous consistency that causes it to move along the direction of impact, resulting in collision with the opposite side of the skull, creating a contrecoup injury. Also possible is a breakdown of the blood-brain barrier (BBB). The BBB is a diffusion barrier that restricts the influx into the brain of molecules only of specific polarities and size (Abbott, Ronnback, & Hansson, 2006; Ballabh, Braun, & Nedergaard, 2004). The BBB is crucial in maintaining the environment for regulated signaling, which can be disrupted post injury (Abbott et al., 2006).

Following trauma to the brain, the central nervous system (CNS) has a unique method of responding to injury: *neuroplasticity*. Neuroplasticity occurs in three distinct phases post injury, presented in *Figure 1*. Phase I begins directly after injury with severe apoptosis and necrosis which continues for 1 – 2 days post injury. Damage to the vascular system from tissue injury can

also lead to impaired cerebral blood flow and ischemia in areas surrounding the impact site, as well as further contributing to the breakdown of the BBB. This vascular compromise can result in inflammation and edema, which represent secondary injury cascades (Price, Wilson, & Grant, 2016). Inflammation represents a particularly key process in this phase. Inflammation constitutes an immune response to compromised tissue by blunt trauma or infection. This immune response is characterized by both pro-inflammatory agents as well as anti-inflammatory agents. Pro-inflammatory markers signal leukocytes to enter the site of injury in order to remove the dead cells through phagocytosis. In the brain selectively, inflammation can additionally cause demyelination through the degradation of the myelin sheath of axons, which can result in a multitude of pathologies (Compston, 1993). Therefore, inflammation can have severe negative long-term implications if not downregulated by anti-inflammatory agents, and thus pro-inflammatory levels could serve as a marker of severity. The rupture of the BBB furthermore can cause leakage of blood cells and serum components into cerebral tissue. This leakage can allow fluid to accumulate in the brain, i.e., edema. These processes can further cause the activation of an immune response, manifesting as inflammation (Morganti-Kossmann, Rancan, Otto, Stahel, & Kossmann, 2001). After TBI, there is also an increase in the number of oxidative-stress markers, such as reactive oxygen species (ROS), and a decrease in antioxidant defense enzymes, such as glutathione (GSH; Ana, Juan Jose, Francisco, & Antonio, 2014). The imbalanced ratio of oxidative-stress markers to antioxidant enzymes leads to increased oxidative stress. Glutamate excitotoxicity also plays an important role in secondary injuries of TBI. Acute stress caused by injury increases extracellular glutamate, which can lead to overstimulation of glutamate receptors. Overstimulation of glutamate receptors leads to an influx of calcium into the cell.

Increased intracellular calcium concentration can lead to a number of processes that cause neuronal damage and death (Yi & Hazell, 2006).

Following apoptosis, an excitatory mechanism initiates *synaptogenesis*, the sprouting of new synapses, and *neurogenesis*, the growth of entirely new neurons. Synaptogenesis and neurogenesis represent the entry into Phase II of neuroplasticity. Synaptogenesis is a complex developmental process that involves synapse formation, the maintenance and stabilization of synapses, and synapse refinement and elimination. Thus, synaptogenesis is a crucial process in both developing new synapses and maintaining the current ones. Neurogenesis is an equally complex process witnessed in all mammals, where functional neurons are produced from their neural precursors, such as stem cells (Ming & Song, 2011). Phase II also recruits new cells, both neuronal and nonneuronal (e.g., glial cells, endothelial progenitors, inflammatory cells) to aid in removing and replacing damaged cells, the formation of gliotic scar tissue, and revascularization (Laskowitz & Grant, 2016). Astrocytes, glial cells that serve as support for neural tissue, undergo reactive astrogliosis in the presence of diseased or damaged tissue (Sofroniew & Vinters, 2010). Astrogliosis constitutes an increase in the number of astrocytes as a result of damage to nearby neurons from injury. Astrogliosis is often implicated in the formation of scar tissue, specifically glial scars, composed of reactive astrocytes and connective-tissue elements, such as proteoglycans, as a mechanical stabilization of CNS tissue (Silver & Miller, 2004; Sofroniew, 2009). Revascularization is, in this case, an endogenous process where increased blood supply is directed to a specific location in order to amend reduced, ischemic, or impaired circulation to an affected organ (Carmeliet, 2003; Tomanek, 2012). Increased cerebral blood flow could be due to greater metabolic demands, increased intracranial pressure, angiogenesis, or vasculogenesis (Kelly et al., 1997; Morgan, Kreipke, Roberts, Bagchi, & Rafols, 2007).

The third and final stage of neuroplasticity (Phase III) constitutes an upregulation in axonal sprouting and other forms of neuritic outgrowth. Axonal sprouting is a means of plastic remodeling. While the damaged axons cannot regenerate, surviving neurons induce outgrowth of new axon collaterals. Sprouting allows for new circuitry to be formed, partial reinnervation, and functional changes (Deller et al., 2006). The neuritic outgrowth similarly refers to the process by which new projections of neurons aid neuron growth. Overall, the entire duration of neuroplasticity is relatively unknown, and through a longitudinal model, timelines may be elucidated (Laskowitz & Grant, 2016).

Applying this tripartite division of the biomechanisms of neuroplasticity, the remainder of this introduction considers the most promising blood biomarkers that may assist physicians with differential diagnosis of TBI. We consider only those biomarkers that are likely to play a role in longitudinal differentiation. The reason for doing so is to better understand which longitudinal biomarkers might best predict neurocognitive decline—a ramification that often results from TBI and presents as the signs and symptoms of many common neurodegenerative diseases. While positron emission tomography (PET) and magnetic resonance imaging (MRI) have been utilized in the diagnosis of TBI, we focus here exclusively on blood biomarkers, as they represent the most cost-effective and least invasive method. Furthermore, PET involves ionizing radiation, which contributes to increased risk of carcinoma. MRI is limiting in view of the exclusion of patients with claustrophobia, pacemakers, or metallic fragments—a common occurrence following shrapnel wounds. Lumbar puncture required for cerebrospinal fluid (CSF) biomarkers is invasive, expensive, and time intensive. While phlebotomy can entail risks of bleeding, infection, and fainting, these risks are rare. Therefore, in the current study, we aim to use the understood neurobiological pathways of neuroplasticity to examine peripheral blood

biomarkers analyzed from serum and plasma in order to determine the presence and potential longitudinal course of TBI applying a minimally invasive, more practical method. Blood biomarkers constitute the most universally applicable procedure for the largest number of patients.

Several proteins have previously been utilized as biomarkers to infer the occurrence of TBI or to guide decisions about the use of further diagnostic brain imaging such as computed tomography (CT). Particularly promising as longitudinal TBI biomarkers are those involved in pro-inflammatory processes. Pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α) and interleukins IL1- β , IL-1, and IL-6 are known to cause cell death and tissue destruction, allowing the first phase of neuroplasticity to take place (Dinarello, 2000; Niesman et al., 2014). Cytokines represent a broad category of small molecules that function in cell signaling. They often are involved in communication between cells in immune responses. Interleukins represent a subgroup of cytokines expressed in leukocytes and, as such, are highly involved in immune response post injury. They have been implicated in cell growth, motility, proliferation, differentiation, and inflammation. IL-6 has also been independently implicated in the revascularization and production of scar tissue present in Phase II of recovery (Galindo et al., 2011; Morganti-Kossmann, Satgunaseelan, Bye, & Kossmann, 2007). The interleukin IL-2 has been found to regulate the number of regulatory T-cells (Tregs). Tregs suppress inflammation, aid neurological functioning in recovery through the reduction of both the BBB disruption and pro-inflammatory responses, and reduce edema, crucial to the revascularization and healing at the end of Phase II of the recovery process (Lowther & Hafler, 2012; Xu, Li, & Jiang, 2013). The up-regulation of Tregs has also been found to degrade the amount of TNF- α and IL1- β , both of which have been implicated in the cell death of Phase I. IL-2 has been found to constitute a

stabilizing factor in blood vessels following TBI and to alleviate excessive inflammation that prevents regeneration (Gao et al., 2017).

Other potentially viable longitudinal biomarkers include the neurosteroids. Neurosteroids represent breakdown products of cholesterol. A schematic of the catabolism of the neurosteroids thought to be most relevant to neuroplasticity is presented in *Figure 2*. The neurosteroids progesterone and estrogen act as agents with neuroprotective functions. Neurosteroid derivatives additionally produce an anti-inflammatory mechanism through the reduction of apoptosis and edema. Through the modulation of aquaporin-4, a water membrane channel protein, they decrease the water content, or edema, in the brain. They furthermore reduce cell death due to the upregulation of levels of the Bcl-2 protein, which is understood to be an anti-apoptotic protein, while decreasing pro-apoptotic protein levels, such as Bax (Robertson et al., 2015). The reduction of cell death and inflammation, therefore, ends Phase I and prompts the regenerative aspect of Phase II. Progesterone is specifically known to be a pleiotropic agent with beneficial effects on Phase II of neuroplasticity, namely reducing cerebral neuroinflammation and excitotoxicity and increasing remyelination (Robertson et al., 2015). Progesterone furthermore reduces excitotoxicity by attenuating the rise in intracellular calcium levels through inhibition of the voltage- and receptor-gated channels (Robertson et al., 2015). Progesterone's reduction of inflammation is due to the suppression of both microglial activation and pro-inflammatory cytokine generation, though the specific mechanism by which it does so is unknown (Robertson et al., 2015). Progesterone and its derivatives have also been implicated as important for myelination after trauma. During myelination after injury, increases are observed in the mRNA for the cytochrome P450_{scc}, which converts cholesterol to pregnenolone, 3 β -hydroxysteroid dehydrogenase, which converts pregnenolone to progesterone, and progesterone receptors

(Robertson et al., 2015). This process of catabolic conversion and receptor proliferation has been found to increase the number of mature oligodendrocytes and the rate of myelin formation (Robertson et al., 2015). Therefore, a known deficit in progesterone is present post TBI.

A similar effect is found for allopregnanolone (ALLO), a twice-reduced metabolite of progesterone (see *Figure 2*). Therefore, similarly to progesterone, ALLO contributes to the end of Phase I leading to Phase II, in addition to actions in Phase III. Specifically, ALLO is involved in reducing neurodegeneration and inflammation. ALLO was found to decrease both the apoptotic DNA fragmentation and the expression of pro-apoptotic proteins, such as Bax (He, Hoffman, & Stein, 2004). The reduction of inflammation might be attributed to the reduction of pro-inflammatory cytokine production, such as TNF- α , and the increase in production of the CD-55 protein, which is a complement convertase inhibitor known to reduce inflammatory processes (VanLandingham, Cekic, Cutler, Hoffman, & Stein, 2007). ALLO is also implicated in neuroregeneration through cell proliferation and decreasing demyelination (Ahmad et al., 2005; Charalampopoulos et al., 2006; He, Hoffman, & Stein, 2004; Naylor et al., 2010; Naylor et al., 2016; Wang, Johnston, Ball, & Brinton, 2005; Xilouri & Papazafiri, 2006). Pregnenolone is yet another neurosteroid that is implicated in the regenerative aspect of neuroplasticity observed in Phase III. Pregnenolone has been found to regulate myelination, as described earlier, and enhance axonal growth and neuritic outgrowth (Flood, Morley, & Roberts, 1992; Koenig et al., 1995; Naylor et al., 2016; Zhu & Glaser, 2008).

Genetic Effects on TBI Recovery

Genetics can influence both a brain's susceptibility to injury following trauma and its method of recovery, including neuronal growth, axonal sprouting, and revascularization

(Houlden & Greenwood, 2006). In particular, apolipoprotein (Apo) E, a 299-amino acid protein transcribed from the APOE gene, plays a significant role in repair, maintenance, and growth of neurons (Houlden & Greenwood, 2006; Verghese, Castellano, & Holtzman, 2011). Its function arises from its role as a cholesterol transporter delivering the molecules to the liver or extrahepatic cells for elimination, thereby maintaining standardized blood cholesterol levels (Li et al., 2015; Mahley & Rall, 2000). ApoE also facilitates the repair of injured cell membranes and neurons, aiding in regrowth (Li et al., 2015; Mahley & Rall, 2000). Two mutant isoforms predominate in the population relative to the ancestral APOE ϵ 4: APOE ϵ 2 and APOE ϵ 3—both of which result in cysteine or arginine amino-acid changes in the APOE gene at positions 112 and 158 (Fullerton et al., 2000; Olsen, Agam, Davis, & Raber, 2012; Verghese et al., 2011). ApoE4 was found to be the most cationic, with the highest charge, differing from ApoE3 by 1 charge unit and ApoE2 by 2 charge units (Mahley & Stanley C. Rall, 2000). The structural difference of ApoE4 compared with ApoE3 and ApoE2 appears to impede its function as a protein. This was seen in its reduced mediation of growth and neural branching, which would represent a strong deficiency in a response to a TBI (Houlden & Greenwood, 2006). Similarly, the APOE- ϵ 4 isoform has been found to be related to attentional deficits, white matter abnormalities, increased risk of AD, and poorer outcomes post mild, moderate, and severe TBI (Houlden & Greenwood, 2006; Lachapelle, Bolduc-Teasdale, Ptito, & McKerral, 2008; Mahley & Rall, 2000; Van Den Heuvel, Thornton, & Vink, 2007). The APOE- ϵ 2 isoform confers a level of neuroprotection against the onset of AD (Farrer et al., 1997).

As genetics have been found to affect neuroplasticity, the biomarkers that have been observed to be involved throughout the process would be expected to be influenced by APOE. The genetic isoform of APOE predetermines premorbid levels of the biomarkers previously

described. Abnormal levels of biomarkers observed as a function of APOE isoform are seen in both Phase I and Phase II of the tripartite model. In Phase I, an abnormal upregulation with ApoE4 occurs for IL-1 β and TNF- α , both implicated in apoptosis and tissue death as noted previously (Huebbe, Jofre-Monseny, & Rimbach, 2009; Lynch et al., 2003; Yin, Zhang, Sun, Mao, & Pan, 2010). Through the increased levels, a greater proportion of cells will die if the patient has the APOE- ϵ 4 isoform. Similarly, an upregulation of IL-6 and IL-2 occurs—implicated in Phase II of the neuroplasticity model (Huebbe et al., 2009; Lynch et al., 2003; Yin et al., 2010). On the other hand, BDNF, implicated in Phase III of neuroregeneration, is decreased in ApoE4 patients. This decrease will lead to less neuron regeneration and greater inflammation compared with ApoE2 and ApoE3 (Álvarez, Aleixandre, Linares, Masliah, & Moessler, 2014; Reverte, Klein, Ratner, Domingo, & Colomina, 2012).

Neurocognitive Impairment

TBI can exert significant effects on cognitive capabilities over time, particularly in selective and sustained attention, processing speed, and verbal memory (Mathias, Beall, & Bigler, 2004). Operationalizing extent of longitudinal neurocognitive decline in combination with biomarker quantification may allow clinicians to better predict the onset of AD and other neurodegenerative disorders following TBI. The Stroop Color-Word Test is the neuropsychological instrument most commonly used to assess selective attention in TBI patients (Ben-David, Nguyen, & van Lieshout, 2011). The Stroop requires the participant to name the colors of ink of words designating colors different from the ink. For example, the word RED is printed in green, and the participant must name “green.” A highly significant difference in speed of performance has been found for the Stroop between controls and participants with TBI

across a multitude of studies (Ben-David et al., 2011). The CVLT-II evaluates memory retrieval, specifically verbal memory. The CVLT-II has been reported to provide a sensitive measure for the assessment of memory deficits in TBI and to relate to TBI severity, specifically for the Long Delay Free Recall subtest (Duchnick, Vanderploeg, & Curtiss, 2002). Assessment of complex visual information processing, including processing speed, has also been found to be an effective tool in evaluating TBI severity and the cognitive decline that follows (Lachapelle et al., 2008). This domain of function can be measured using the WAIS-III Symbol Search subtest, which incorporates visuospatial complexity as well as processing speed.

In summary, following TBI, a cascade of neurobiological events mediating neuroplasticity occurs, as well as neurocognitive changes that may represent harbingers of neurodegenerative dementia. Thus, it was hypothesized that 1) blood biomarkers will be significantly different between those with TBI and those without, 2) a significant difference will be observed in the extent of cognitive decline from baseline to follow-up between TBI positive and TBI negative subjects, and 3) a negative relationship will be observed between biomarker levels and extent of cognitive decline. By examining the association between the presence of these blood biomarkers and cognitive decline, we will gain a better understanding of their predictive value and therefore the differential mechanisms of recovery post TBI. This work will facilitate improved methods of diagnosing TBI, enhance prognostic determination of likely TBI outcomes, and promote development of targeted therapies based on the levels and neurobiological and physiological actions of specific blood biomarkers.

Methods

A sample of research volunteers was assessed at the Mid-Atlantic Research, Education, and Clinical Center (MIRECC) of the Durham Veterans Affairs Medical Center (VAMC). This study was approved by the Durham VAMC Institutional Review Board (IRB). Subjects were included in the study if they were current or former military personnel serving any time after September 11, 2001. This study constitutes a longitudinal follow-up of baseline data acquired between 2006 and 2008. Baseline data collection is described followed by the longitudinal assessment conducted for this study.

Baseline Assessment

At baseline, a large database repository consisting of demographics, medical information, deployment information, and descriptions of any TBIs that occurred throughout the participants' lifetime was created. A phlebotomy to test for prominent biomarkers was also obtained. A short time following the repository visit, a 6-hour battery of 21 neurocognitive tests was administered. Data from the repository, phlebotomy, and neurocognitive tests were used to compare with the longitudinal data collection of the present study to examine for biomarker prediction of cognitive change over the 10 – 12-year interval since the baseline measurements.

Longitudinal Assessment

Subjects

A target subset of the original baseline sample of subjects ($N=31$) was selected to return for repeat longitudinal testing. This subset of participants was largely middle-aged, with an average age of 47.9 ± 8.9 years. The sample was 54.6% Caucasian and 45.2% African American.

Fifteen subjects had a self-reported history of TBI. Sixteen subjects were negative for TBI, serving as controls. All were male.

Twenty-nine participants with a permanent address within 150 miles were mailed an IRB-approved letter describing the study, followed by attempts by the present investigator to contact them via telephone.

Materials

Five primary categories of data were examined comparing baseline to the 10- to 12-year longitudinal follow-up. These categories included information on the unique attributes of TBI and individual TBI history, blood biomarkers, APOE, and neurocognition.

TBI Attributes and History. The McCormick TBI Interview, developed in house, was administered by trained personnel at the baseline assessment. This interview obtains information about the individual's TBI history in order to document the frequency of TBI occurrences throughout an individual's lifetime and the characteristics of each individual injury. For each individual injury reported by a participant, the duration of symptoms is assessed, including length of loss of consciousness (LOC) and post-traumatic amnesia (PTA). This information informs classification of TBI severity. LOC was classified as none, <1 minute, 1 – 30 minutes, 30 minutes – 6 hours, 6 hours – 24 hours, and >24 hours. If an individual reported no LOC, he/she was able to report whether or not he/she was “dazed and confused” in a separate item. PTA was classified by none, < 1 hour, 1 – 24 hours, 24 hours – 7 days, and >7 days. For the current study, a history of TBI was defined as present if any period of LOC, becoming “dazed

and confused,” or any duration of PTA was endorsed. Participants denying all three indices of altered consciousness or memory were classified as controls.

The Computerized Patient Record System (CPRS) electronic medical record from the Durham VAMC was used to confirm all data regarding medical conditions and injuries.

Blood Biomarkers. Phlebotomies were performed at baseline by a trained phlebotomist researcher within the MIRECC. Approximately 15 mL of blood was drawn from each participant between 11:00 a.m. and 2:00 p.m. to control for diurnal variation.

Cognition. Change in neurocognitive performance from baseline to longitudinal assessment was evaluated by a battery of 17 tests repeated from the baseline assessment. The 6-hour baseline battery consisted of: Grooved Pegboard, University of Pennsylvania Smell Test, Behavioral Dyscontrol Scale, Brief Visuospatial Memory Test-Revised, Wechsler Test of Adult Reading, WAIS-III (Digit Symbol-Coding, Similarities, Block Design, Symbol Search, Letter-Number Sequencing subtests), Stroop Color-Word Test, CVLT-II, Controlled Oral Word Association, Finger Oscillation Test (Finger Tapping), Trail Making Test, Continuous Performance Test, Paced Auditory Serial Addition Test, Auditory Consonant Trigrams, Rey-Osterrieth Complex Figure, Russell Figural Fluency Test, and Word Memory Test. The longitudinal follow-up included a reduced, 3- to 4-hour battery consisting of a subset of the original tests: Grooved Pegboard, an abbreviated version of the University of Pennsylvania Smell Test, Behavioral Dyscontrol Scale, Brief Visuospatial Memory Test-Revised, Wechsler Test of Adult Reading, WAIS-III (Digit Symbol-Coding, Similarities, Block Design, Symbol Search, Letter-Number Sequencing subtests), Stroop Color-Word Test, CVLT-II, Finger Oscillation Test (Finger

Tapping), Trail Making Test, Continuous Performance Test, Russell Figural Fluency Test, and Word Memory Test. In addition, two tests were added from the WAIS-III (Digit Span and Matrix Reason), and Controlled Oral Word Association was replaced by the FAS Test, which has identical procedures but uses the letters F, A, and S rather than C, F, and L. Two separate test orders were devised for counterbalancing across subjects to control for test order and/or fatigue. This battery was reduced from the original baseline battery based on prior assessment of the neurocognitive tests most discriminating of cognitive impairment and the possibility that the prior 6 hours of testing may have been unduly taxing on the participants. The entire battery of tests was required in order to conduct a valid administration, as tests of delayed memory are administered in which the period of delay must be filled with tests that draw upon the opposite modality (i.e., verbal-memory tests are interpolated with visual material and visual-memory tests are interpolated with verbal material). Analyses were confined to three principal test measures considered to be most promising based on the prior literature review: WAIS-III Symbol Search, Stroop Color-Word Test, and CVLT-II Long-Delay Free Recall.

WAIS-III Symbol Search: The WAIS-III Symbol Search presents two groups of symbols: a pair of two symbols on the left and a group of five symbols on the right. The subject is required to draw a line through a box labeled “YES” if one of the symbols on the left is present in the group of symbols on the right. The subject is instructed to draw a line through a box labeled “NO” if none of the symbols on the left are present in the group of symbols on the right. The dependent measure is the number of symbols correctly matched in 120 secs, with the number of errors subtracted from the total score (Wechsler, Coalson, & Raiford, 1997).

Stroop Color and Word Test: The Stroop Color and Word Test is one of the oldest and most widely used tests in psychology for examining attention and response inhibition (Stroop, 1935). Subjects perform 3 tasks as quickly as possible: (a) read words in black ink that are the names of colors (i.e., color words), (b) name the ink color of standard stimuli (e.g., “XXX” in green, blue, and red ink), and (c) name the color of the ink in which noncongruent color words are printed (e.g., say “red” when the word “green” is printed in red ink). The number of names correctly provided in 45 seconds constitutes the dependent measure. The effects of response inhibition are indicated by subjects' slower RT when naming the color of the ink of noncongruent color words than when reading words that are the names of colors or naming the color of meaningless stimuli. The test takes approximately 4 minutes to administer.

CVLT-II: The CVLT-II examines several aspects of verbal learning, organization, and memory, as well as processes by which learning occurs (Becker & Lim, 2003). Subjects listen to a list of 16 words falling into 4 categories and must then recall them (list A), and after 5 trials they are provided with a new list of 16 words that they must recall (list B). Subjects then recall the original list (A) both immediately and after a delay of 20 minutes. Subjects are also provided with categorical cues after both of these trials, and at the end of the test they are provided with a yes-no recognition trial and then a forced-choice recognition trial. The test requires 20 minutes to administer, not including the interpolation interval. The dependent measure is the number of words correctly recalled.

Procedure

Phlebotomy. Phlebotomy procedures, conducted at the baseline visit, asked the participant to identify the preferred arm for a needle stick. An alcohol cleansing swab was then applied to the prospective area of the needle stick. Fifteen mL was drawn and placed in a red-topped tube. A bandage was applied to the phlebotomy site. The blood was then centrifuged to separate plasma from serum.

Blood Biomarker Analysis. The neurosteroid blood biomarkers were analyzed and quantified using gas chromatography (GC)/mass spectrometry (MS), preceded by high performance liquid chromatography (HPLC) with minor modifications. One modification was that the electron impact mode was utilized rather than negative ion chemical ionization, as reported previously (Marx et al., 2006; Sripada et al., 2013). Samples were extracted three times in ethyl acetate prior to HPLC. Mean intra-assay coefficients were 1.6% for pregnenolone and 3.9% for ALLO. The sensitivity of this methodology is 1 pg for each neurosteroid. All of the glassware used was salinized. Peaks with the ratio of signal to noise 5:1 were integrated. Due to technical difficulties, data were lost for one subject.

For cytokines and other small molecules, commercially available ELISA kits were procured from ThermoFisher Scientific catalogue and utilized per manufacturer's directions. Fifteen mL of serum were diluted 5 fold with 4 volumes of water.

Genotyping. Genomic DNA was isolated from serum blood samples using standard salting techniques, according to Kimbrel and colleagues (Kimbrel et al., 2015). APOE genotypes were

determined utilizing the commercially available TaqMan® genotyping assays procured from ThermoFisher Scientific, following manufacturer's directions. Genotype frequencies in the total sample were as follows: $\epsilon 2/\epsilon 3$ ($n=4$), $\epsilon 2/\epsilon 4$ ($n=2$), $\epsilon 3/\epsilon 3$ ($n=15$), $\epsilon 3/\epsilon 4$ ($n=3$), and $\epsilon 4/\epsilon 4$ ($n=1$).

Neurocognitive Assessment. Neurocognitive testing was administered at longitudinal follow-up by the current investigator in accordance with the participant's counterbalanced assignment. Participants were provided with 10-min rest breaks as needed. At the conclusion of testing, the participants were provided with forms to complete to receive reimbursement of \$150 plus travel costs for compensation.

Statistical Analysis. Statistics were computed in SAS version 9.4 (SAS Institute Inc, Cary, NC). Univariate statistics were used to describe the sample. Participants were classified by TBI group status as either positive (+TBI) or negative (-TBI). A repeated-measures general linear model (GLM) analysis was used to examine baseline blood biomarker levels and changes in neurocognitive performance on the three cardinal dependent measures from Baseline to Longitudinal follow up. TBI group status was entered as a dichotomous class variable.

Results

Baseline

Neurosteroids

The effect of TBI status on the neurosteroids as a group was significant, $F_{(2,27)}=4.32$, $p=0.047$. The effect of the repeated measure was highly significant, $F_{(2,27)}=77.49$, $p<.0001$.

Furthermore, the interaction between TBI status and neurosteroid levels was significant

$F_{(2,56)}=4.32, p=0.0179$. Examination of the individual neurosteroids revealed a significant effect for pregnenolone $F_{(2,28)}=4.46, p=0.0438$, as seen in *Figure 3*. Participants with TBI had significantly lower values (380.6 ± 201.2 pg/mL) than those without TBI (539.2 ± 207.3 pg/mL). For ALLO, although the mean for participants with TBI (55.0 ± 44.9 pg/mL) was lower than for participants without TBI (68.3 ± 32.6 pg/mL), results were not significant, $F_{(1,28)}=0.81, p=0.3754$. Similarly, progesterone did not have a significant effect, $F_{(1,28)}=0.02, p=0.8943$. Participants with TBI-positive status had very similar values of progesterone (0.15 ± 0.14 pg/mL) compared with those without a history of TBI (0.15 ± 0.06 pg/mL).

Cytokines

Although the effect of the repeated measure was highly significant, $F_{(1,29)}=421.56, p < .0001$, the effect of TBI status on the cytokines as a group was not significant, $F_{(1,29)}=0.15, p=0.7058$. Likewise, the interaction between TBI status and cytokine levels was not significant, $F_{(1,29)}=0.06, p=0.8037$. Examination of the individual cytokines revealed a lack of significance for both TNF- α and IL1- β . Participants with TBI had a slightly smaller mean level of TNF- α (2.3 ± 0.54 pg/mL) compared with those without TBI (2.38 ± 0.75 pg/mL); however, TNF- α levels were not significant with respect to TBI status, $F_{(1,29)}=0.10, p=0.7158$. Similarly, although participants with TBI-positive status had slightly lower means of IL1- β (0.04 ± 0.05 pg/mL) compared with those without a history of TBI (0.06 ± 0.06 pg/mL), IL1- β levels were not significant in relation to TBI status, $F_{(1,29)}=0.80, p=0.3785$.

Interleukins

Although the effect of the repeated measure was highly significant, $F_{(1,29)}=36.76$, $p<.0001$, the effect of TBI status on the interleukins as a group was not significant, $F_{(1,29)}=0.73$, $p=0.4012$. Furthermore, the interaction between TBI status and interleukin levels was not significant, $F_{(1,29)}=1.60$, $p=0.2156$. Examination of the individual interleukins revealed a significant effect for IL-2, $F_{(1,29)}=11.46$, $p=0.0021$. Participants with TBI had a significantly lower mean for IL-2 (0.009 ± 0.02 pg/mL) compared with those without TBI (0.06 ± 0.06 pg/mL), as seen in *Figure 4*. On the other hand, although participants with TBI had a slightly higher mean for IL-6 (0.86 ± 0.81 pg/mL) compared with those without TBI (0.62 ± 0.24 pg/mL), examination of IL-6 individually revealed a lack of significance, $F_{(1,29)}=1.12$, $p=0.2976$.

APOE

The interaction between APOE and neurosteroid levels was not significant ($p=0.5273$). Likewise, the interaction between TBI status and APOE was not significant either ($p=0.1104$). However, the triple interaction between TBI, neurosteroids, and ApoE status was marginally significant, $F_{(2,52)}=2.60$, $p=0.0835$. No significance was found for the omnibus statistic for ALLO ($p=0.6275$) or progesterone ($p=0.8702$). However, it was marginally significant for pregnenolone, $F_{(3,26)}=2.63$, $p=0.0716$. Those who had an APOE isoform $\epsilon 4$ with a history of TBI had notably lower mean amounts of pregnenolone (313.0 ± 80.3 pg/mL) than those without a history of TBI (708.5 ± 371.2 pg/mL). This was also seen in those without an APOE- $\epsilon 4$ isoform but to a lesser degree, with those with TBI having a lower mean (395.1 ± 218.1 pg/mL) than those without a TBI (488.4 ± 119.3 pg/mL). Those with an APOE- $\epsilon 4$ isoform and a TBI history had less mean pregnenolone (313.0 ± 80.3 pg/mL) than those without an APOE- $\epsilon 4$ isoform and with a positive TBI history (395.1 ± 218.1 pg/mL) as well.

Longitudinal Follow-up

Participants

Ten participants were successfully recruited to return for longitudinal testing. *Table 1* presents characteristics of each participant in the longitudinal sample. Out of the remaining 21, one participant was deceased, four refused participation for various reasons including desires to “never go down that road [of thinking about their experience in the military] again,” and 10 were unreachable due to inaccurate contact information in the CPRS medical records. The average age of the participants was 49.8 ± 5.9 years old, and 60% were Caucasian and 40% were African American. Out of the 10 participants, 5 were expected to have a history of TBI and 5 were expected controls with no history of TBI, according to the McCormick TBI Interview and CPRS. However, upon return for longitudinal testing, two of the controls revealed past TBIs that they had been “ashamed” to acknowledge to medical personnel at the time. However, due to recent concerns of loss of memory and cognitive functioning, they revealed the true nature of their TBI status. Thus, our final sample contained 7 TBI-positive veterans and 3 veterans with no history of TBI.

Neurocognition

The triple interaction between change in cognition level, TBI status, and specific test was marginally significant, $F_{(2,12)}=3.63$, $p=0.0569$. Due to one subject choosing to discontinue testing, the CVLT-II longitudinal results have one less subject, which could be responsible for the marginal significance. To maximize statistical power in view of the already-low sample size, tests were subsequently divided into two groups for attentional characterization using the Stroop

and WAIS-III Symbol Search measures and memory capabilities using the CVLT-II Long Delay Free Recall data.

Attentional Results. The effect of TBI status on change in cognition was highly significant for the attentional measures, $F_{(1,6)}=9.79$, $p=0.0166$, as seen in *Figure 5*. When considering individual tests, Symbol Search at baseline was not significant between those with a history of TBI and those without a history ($p=0.5117$). The means were relatively similar for those with TBI (33.7 ± 8.3) and those without a history of TBI (37.3 ± 4.9). Similarly, TBI status did not affect performance on the Stroop at baseline ($p=0.5800$). The means for those with TBI (43.0 ± 5.5) were roughly similar to those without TBI (42.3 ± 6.0). However, both tests suggested a separation between groups at the longitudinal time point, and both had similar directionalities. Specifically, the effect of TBI status on Symbol Search was marginally significant, $F_{(1,7)}=4.67$, $p=0.0674$. Those with a history of TBI had lower mean performance (27.0 ± 7.7) than those without (38.3 ± 6.7). Similarly, the effect of TBI status on the Stroop was marginally significant, $F_{(1,7)}=3.70$, $p=0.0957$. Those with TBI (31.8 ± 7.2) had lower scores than those without a TBI (42 ± 8.2) as well, indicating that with a larger sample size, these effects would have most likely been significant.

Memory Results. The effect of TBI status on change in cognition was not significant for the memory measure, $F_{(1,6)}=0.95$, $p=0.3673$. TBI status did not affect the CVLT-II measure at baseline ($p=0.4516$) or longitudinal follow-up ($p=0.3283$). However, their means do indicate similar directionality as the two attentional tests examined. At baseline, those with a TBI ($11.4 \pm$

1.7) had a lower mean (12.7 ± 2.9) than those without history of TBI. At longitudinal follow-up, those with a TBI had a lower mean (10.0 ± 3.4) than those without a TBI (12.7 ± 3.5) as well.

Relationship between Blood Biomarkers and Longitudinal Decline in Cognition

Correlation coefficients computed between the two significant blood biomarkers between TBI and controls revealed negative coefficients for all six analyses. The relationship between Stroop decline and pregnenolone was -0.40. Its relationship with IL-2 was -0.18. The relationship between CVLT-II decline and pregnenolone was -0.40. Its relationship with IL-2 was -0.40. The relationship between Symbol Search and pregnenolone was -0.57, while its relationship with IL-2 was -0.45. Although the magnitude of the coefficients reflected small to medium effect sizes, results did not achieve statistical significance due to the reduction in the already-low sample size upon confining analyses to only those subjects with a history of TBI.

Discussion

TBI has become a pervasive issue in the military, especially with the advent of the IED. Due to the impact to the brain, the neurodegeneration that results from the TBI can manifest in significant cognitive decline. However, one fundamental clinical issue is that without direct observation by medical personnel at the time of the event or the subsequent application of neuroimaging, TBI must be self-reported, which can be problematic. However, blood biomarkers could serve as a minimally invasive method for assessing the presence of TBI and the longitudinal implications of the injury. The present work represents one of the only investigations to our knowledge that explores this possibility in military veterans. Despite the small sample size of the present study, some of the biomarkers and neurocognitive tests

investigated yielded statistical significance when comparing positive versus negative TBI status, demonstrating the promise of this methodological approach.

When considering the significance found in the interaction between TBI status and neurosteroids supporting hypothesis 1, there are many critical factors to account for. The first is that despite the disparate level of significance encountered for individual neurosteroids, the group of neurosteroids as a whole was significant due to all of the individual neurosteroid levels exhibiting lower values in those with TBI than those without a history of TBI. This is noteworthy because it indicates the directional effect of neurosteroid levels based on TBI status. Further, it indicates that possibly with a larger sample size, the results may have been significant for each individual neurosteroid as well. However, there is also another consideration that could have affected these results. Progesterone is specifically known to have beneficial effects on Phase II of neuroplasticity, reducing neuroinflammation and excitotoxicity (Robertson et al., 2015). Similarly, ALLO has been shown to decrease apoptosis and apoptotic-related proteins (He, Evans, Hoffman, Oyesiku, & Stein, 2004). These neurosteroids are both strongly implicated in Phase I and Phase II of neuroplasticity. On the other hand, pregnenolone has been found to affect remyelination, enhancing axonal growth and neuritic outgrowth (Flood et al., 1992; Koenig et al., 1995; Naylor et al., 2016; Zhu & Glaser, 2008). This is indicative of Phase III of neuroplasticity. Thus, the particular time point in the neuroplasticity timeline may affect the biomarker levels. These phlebotomies could have been performed during the third phase and therefore affected the results of the markers. This would explain the overall directional decrease compared with non-TBI subjects although the levels approached normalization to the point that they were not statistically significant.

The analyses examining APOE were also interesting. As APOE status has been highly implicated in the development of, and susceptibility to, AD, its relationship to TBI and the long-term consequences associated with blood biomarkers was a novel concept that has not been studied to our knowledge. However, APOE status was found to be marginally significant in its relationship to neurosteroid levels based on TBI status. These results indicate that with the APOE-ε4 isoform, associated with attentional deficits and poorer outcomes post TBI, there is a relationship with lower neurosteroid levels, which are also associated with cognitive dysfunction (Gatson et al., 2016; Houlden & Greenwood, 2006; Mahley & Stanley C. Rall, 2000; Van Den Heuvel et al., 2007). Thus, those that are at highest risk of long-term cognitive deficiencies are those with a history of TBI and the APOE-ε4 isoform, perhaps influenced by the relationship of APOE to the biomarkers studied. This could be a key factor for clinicians routinely encountering patients with TBI to consider assessing. This finding is especially telling, as its significance would most likely increase with a larger sample size, which was unfortunately a limitation of our study.

The predominant lack of significance found in the cytokines and the interleukins was an important finding as well. This is because it suggests that prolonged inflammation and cell death is not a significant issue in those with TBIs compared with those without a history of TBI. This finding may have been further affected by the fact that the blood analysis was conducted often several years post TBI, and thus may have skewed results towards the blood biomarkers that would be present in the later stages of neuroplasticity. However, this supports our concept of a precise timeline of neuroplasticity that upregulates and downregulates blood biomarkers based on their function. This is particularly telling, as IL-2 was a significant biomarker distinguishing between TBI and non-TBI subjects. This was critical because much like progesterone, IL-2 is

primarily functioning in the later stages of neuroplasticity in the reduction of inflammation and neural regeneration (Xu et al., 2013). On the other hand, while not significant, IL-6 did have directional differences with a higher mean in those with a history of TBI. IL-6 has been implicated in cell death and inflammation, similar to the cytokines (Galindo et al., 2011; Morganti-Kossmann et al., 2007). Thus, this could further support the supposition that the phlebotomy occurred approximately during the third stage of neuroplasticity, thus reducing the level of significance observed for the biomarkers, such as the cytokines, that would be highly prevalent in the first stage. In order to confirm this possibility, a larger sample size would be required as well as a consistent date since the TBI event.

The high level of significance observed for the effect of TBI status on visual-attentional tests of longitudinal change in cognition is highly informative, thus supporting hypothesis 2. It demonstrates the cognitive decline associated with TBI that has occurred over the last decade for many of the veterans. However, interestingly enough, the lack of significance in the cognitive decline in relation to TBI status in the assessment of memory suggests a non-hippocampally related deterioration. Rather, the lack of significance in the difference in memory tests, combined with the observed attentional decrements, suggests issues with encoding memories, rather than retrieval of the memory itself once formed. Thus, the deterioration, as attentional in nature, may be primarily referable to injury to the frontal lobe. This is particularly relevant, as the frontal lobes are often the site of TBI, especially in IED blasts and motor vehicle accidents, which make up the majority of the sources of TBI in our sample (Stuss, 2011). This interpretation is further supported by findings that those with frontal lobe damage have been found to demonstrate a significantly worse learning index compared with those with temporal lobe deterioration or controls (Schraegle, Nussbaum, & Stefanatos, 2016). The difference, however, is that the CVLT-

It also utilizes the hippocampus for retrieval. Thus, the data suggest that the hippocampus is still principally intact for many of the veterans, as the findings reflected a lack of significant difference in free recall following a delay in encoding between those with TBI and those without a history of TBI. The lack of hippocampal deterioration could be due to the fact that none of the participants in our sample had reached the average assumed age of AD onset, at 65 (Katzman, 1976). Furthermore, the hippocampus resides in the medial portion of the brain, whereas the frontal lobes are distal. The physics of rotation, therefore, would cause the hippocampus to be displaced less than the frontal lobes after being subjected to rotational forces. Furthermore, the frontal lobes are adjacent to the skull, which increases the potential for damage. Furthermore, studies using PET scans have found that at the earliest stages of the disease, AD is marked by impairment of the frontal operculum in the frontal lobe, which then extends out to the temporoparietal cortices in more advanced stages of the disease (Berti, Pupi, & Mosconi, 2011; Perneczky, Diehl-Schmid, Pohl, Drzezga, & Kurz, 2007). This interpretation is further strengthened by the findings that impaired executive function and visuospatial abilities were strong predictors of the conversion from MCI to AD over time (Anchisi, Borroni, Franceschi, & et al., 2005; Chapman et al., 2011). Notably, the Stroop and Symbol Search tests rely strongly on executive control and visuospatial abilities. While we did not test for MCI, our findings suggest that many of the subjects with TBI could be entering into early stages of MCI.

The negative relationship between blood biomarker levels and longitudinal cognitive decline was tentatively supportive of hypothesis 3. All six correlations were negative, with small to moderate effect sizes ranging between -0.18 and -0.57; however, results were not statistically significant. Due to the very small sample size ($n = 4 - 6$), based on selective data loss or willingness to fully complete the study, the burden of achieving statistical significance was

prohibitive. But in view of the notable effect sizes, it is believed that with a larger sample size, statistical significance would be obtained. Thus, future studies should explore this relationship with a large sample.

Several limitations to this paper are important to acknowledge. A major limitation of this study was its very limited sample size. While efforts were made to recruit a larger sample N , many had relocated and were lost to follow-up in the 10 or more years since baseline, and it proved far more difficult than at first anticipated to encourage veterans to return for longitudinal assessment. This limitation warrants a future study with a far greater sample size, hopefully supporting our results as well as revealing findings that we had not observed. There is great promise in utilizing blood biomarkers to predict changes in cognition and establishing them as a diagnostic standard for evaluating future TBIs.

A second limitation of this study was the inability to conduct longitudinal blood assays. While blood was collected, it proved infeasible to repeat the assays for a longitudinal follow-up examination. While this approach would have been most effective in detailing the change in biomarkers affecting cognition over time, we were limited to investigating how baseline blood biomarkers predicted cognitive changes. Given more time and resources, studying how the blood biomarker levels vary over time would provide important insights into their relationship to recovery, timeline of recovery, how subsequent TBIs affect the neuroplasticity model, and how they affect longitudinal wellbeing.

TBI can manifest differently as a result of the specific mechanism of trauma. Differences could derive from directionality of impact, severity, mechanism of injury (e.g., BINT vs. blunt trauma), or differences in medical care post injury, for example, use of extreme sedation in the emergency department. As such, the variations in these mechanisms could differentially affect

the way in which biomarker levels reflect injury and the resulting susceptibility to neurocognitive decline.

Individual differences are also likely to have an impact on biomarkers and have not been considered. For example, natal sex has been found to have effects on neurosteroid levels as well as some of the aforementioned protein markers. The endogenous levels of certain neurosteroids are inherently higher in females than in males. This difference could account for the fact that greater ischemic sensitivity is present in males compared with females and that females, though receiving an equivalent insult, have better functional outcomes and fewer cerebral lesions than their male counterparts due to the neuroprotective nature of neurosteroids (Vagnerova, Koerner, & Hurn, 2008). IL-6 levels are similarly significantly different between the sexes. IL-6 was found to be higher in males than in females studied post injury (Sperry et al., 2008). However, IL-1 β and TNF- α were found to be equivalent between males and females (Sperry et al., 2008). Therefore, in future studies, natal sex must be incorporated in order to better understand the impact that injury exerts on these biomarker levels differentially in relation to the sexes.

An additional demographic variable that exerts effects on blood biomarkers is age. While once again confounded by sex, neurosteroid levels decrease with increasing age. The discrete hormonal changes that occur for women post menopause could create confounds if the interaction between age and sex is not taken into account. That is, past a certain age, women no longer produce ovarian progesterone or estrogen, lowering the amounts of the neuroprotective neurosteroids, sometimes below the levels of men. These hormonal discrepancies could underlie evidence that the outcome of an ischemic event is worse in older women than in their male counterparts (Wei & Xiao, 2013). With age also comes a decrease in the production of many other biomarkers and weaker immune responses that could affect biomarker levels and

neuroplasticity responses to injury post trauma. Similarly, age affects other protein biomarkers such as S100B, which is significantly lower in newborns than in healthy adults (Dadas, Washington, Marchi, & Janigro, 2016). Yet, the production of serum IL-6 *increases* with age (Maggio, Guralnik, Longo, & Ferrucci, 2006). Therefore, similar to natal sex, some biomarkers are differentially affected by age, and age should therefore be controlled for in order to fully understand what levels of biomarkers are to be expected post injury. As AD represents a disorder of aging, mechanisms of senescence may be particularly sensitive to biomarker levels during certain decades of life and should be further studied. Similarly, while some studies have found that women are more likely to develop AD, others have found little to no sex differences in the disorder (Fratiglioni et al., 1991; Launer et al., 1999; Prencipe et al., 1996).

One study found that ethnicity affected basal levels of biomarkers, specifically S100B. Darker skin tone, specifically in black or African American participants, was associated with higher levels of S100B compared with lighter-skinned participants, including Caucasians, Hispanics, Asians, Native Americans, etc. (Dadas et al., 2016). Another biomarker found to possibly be affected by ethnicity was the genetic influence on APOE isoforms. The APOE ϵ 4-AD association was found to be weaker among African Americans and Hispanics, while stronger in Japanese subjects compared with Caucasian subjects (Farrer et al., 1997).

Another possibly influential variable moderating biomarker levels is lifestyle, particularly smoking, obesity, and sleep deprivation. Smoking has been found to affect levels of neurosteroids specifically. It has been found that smoking lowers levels of dehydroepiandrosterone (DHEA), ALLO, progesterone, and pregnenolone (Iancu et al., 2007). S100B has been found to increase in serum levels with acute sleep deprivation (Benedict et al., 2014). Similarly, DHEA and other neurosteroids have been found to manifest increased plasma

levels in obese patients compared with healthy non-obese controls (Monteleone et al., 2003). Future studies of biomarkers must therefore acquire thorough documentation of lifestyle variables in order to better account for levels attributable to TBI rather than extraneous confounds.

Lastly, another potential confound could include comorbid disorders and diseases, both psychiatric and physical. These could include diabetes, PD, frontotemporal lobar degeneration, and multiple sclerosis (Esopenko & Levine, 2015). All of the mediators of inflammation such as TNF- α , IL-1 β , IL-6, IL-2, and other chemokines have been implicated in both forms of diabetes (Kristiansen & Mandrup-Poulsen, 2005). Ensuring that these participants do not also manifest these conditions, or controlling for them statistically, is critical due to their potentially confounding effects on the biomarkers studied.

Future work, therefore, has much to accomplish in this area of science. While studies controlling for all of the above limitations are necessary, there are other factors and studies that should be explored as well. A study that examines biomarker levels in equal temporal increments across a longitudinal time span would allow for enhanced understanding of how long the levels of some of these biomarkers persist and how they vary over time. One such study could track biomarker changes each week or similarly frequent intervals post trauma in order to better understand the timing of the neuroplasticity model, especially if recruiting mild, moderate, and severe TBI participants, so as to understand how severity of injury affects this timeline. Such studies would provide an enhanced understanding of both their timelines and the role they play in the process of neuroplasticity. This approach would further elucidate many of the questions of significance posed by the present study. One could understand whether cytokines, ALLO, and progesterone play a more significant role earlier in the neuroplasticity model, or whether they are

not significantly incorporated, by means of a timeline-based longitudinal study. Examining the effects of severity of injury on the levels of biomarkers may furthermore lead to a method of diagnosing TBI severity through blood biomarker quantification, as well as enhancing prognosis and further elucidating the pathogenesis of AD and other types of neurodegeneration that can follow TBI.

Future science should also relate biomarkers to MRI findings in order to understand their relationships to morphological changes that could occur in the brain post injury. For example, the findings showing ALLO reductions in the prefrontal cortex and APOE- ϵ 4-related reductions in temporal cortex of postmortem AD brains in comparison with controls is intriguing in suggesting localization of biomarker levels (Naylor et al., 2016). Further work in this area could facilitate improved understand of the effects of TBI despite different biomarker profiles, outcomes, and differential entrance into the neuroplasticity phases. This approach could also help to understand the role of the specific trajectory of acceleration-deceleration damage to the brain and how it affects biomarker levels, as well as possible relationships between biomarker profiles and regional differences in neuroplasticity.

Neurosteroids have potential as therapeutic agents for TBI and recovery attributable to neuroplasticity mechanisms. ALLO has been found to be reduced in patients with posttraumatic stress disorder, thereby serving as a therapeutic agent in treatment supplementation (Naylor et al., 2016; Pinna & Rasmusson, 2012; Rasmusson et al., 2006). Although the present study did not find differences in progesterone between subjects with and without TBI, a similar deficit has been reported in progesterone post TBI, making it a potentially effective therapeutic agent as well. While the neurosteroids, including ALLO, progesterone, and estrogen are all implicated as possible exogenous therapeutic agents, little is known about utilizing protein biomarkers, which

may have low levels following TBI, as exogenous therapeutic agents. Such investigation may be especially informative soon after TBI, while the BBB is still damaged.

By examining how the presence of these blood biomarkers predicts cognitive decline starting with MCI and proceeding to AD, we will acquire a better understanding of their neurobiological functions and therefore the differential mechanisms of neuroplasticity post TBI and their contribution to the declaration of AD. This understanding will allow for targeted therapies and better methods of diagnosing TBI and understanding and predicting future ramifications of the TBI.

Works Cited

- Abbott, N. J., Ronnback, L., & Hansson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*, 7(1), 41-53.
- Ahmad, I., Lope-Piedrafita, S., Bi, X., Hicks, C., Yao, Y., Yu, C., . . . Erickson, R. P. (2005). Allopregnanolone treatment, both as a single injection or repetitively, delays demyelination and enhances survival of Niemann-Pick C mice. *Journal of Neuroscience Research*, 82(6), 811-821. doi:10.1002/jnr.20685
- Álvarez, A., Aleixandre, M., Linares, C., Masliah, E., & Moessler, H. (2014). Apathy and APOE4 are associated with reduced bdnf levels in alzheimer's disease. *Journal of Alzheimer's disease : JAD*, 42(4), 1347-1355. doi:10.3233/JAD-140849
- Ana, R.-R., Juan Jose, E.-G., Francisco, M.-C., & Antonio, C.-V. (2014). Oxidative Stress in Traumatic Brain Injury. *Current Medicinal Chemistry*, 21(10), 1201-1211. doi:<http://dx.doi.org/10.2174/0929867321666131217153310>
- Anchisi, D., Borroni, B., Franceschi, M., & et al. (2005). Heterogeneity of brain glucose metabolism in mild cognitive impairment and clinical progression to alzheimer disease. *Archives of Neurology*, 62(11), 1728-1733. doi:10.1001/archneur.62.11.1728
- Ballabh, P., Braun, A., & Nedergaard, M. (2004). The blood-brain barrier: an overview: Structure, regulation, and clinical implications. *Neurobiology of Disease*, 16(1), 1-13. doi:<https://doi.org/10.1016/j.nbd.2003.12.016>
- Bass, C. R., Panzer, M. B., Rafaels, K. A., Wood, G., Shridharani, J., & Capehart, B. (2012). Brain Injuries from Blast. *Annals of Biomedical Engineering*, 40(1), 185-202. doi:10.1007/s10439-011-0424-0
- Becker, S., & Lim, J. (2003). A computational model of prefrontal control in free recall: strategic memory use in the California Verbal Learning Task. *Journal of Cognitive Neuroscience*, 15(6), 821-832.
- Ben-David, B. M., Nguyen, L. L. T., & van Lieshout, P. H. H. M. (2011). Stroop Effects in Persons with Traumatic Brain Injury: Selective Attention, Speed of Processing, or Color-Naming? A Meta-analysis. *Journal of the International Neuropsychological Society*, 17(2), 354-363. doi:10.1017/S135561771000175X
- Benedict, C., Cedernaes, J., Giedraitis, V., Nilsson, E. K., Hogenkamp, P. S., Vågesjö, E., . . . Schiöth, H. B. (2014). Acute Sleep Deprivation Increases Serum Levels of Neuron-Specific Enolase (NSE) and S100 Calcium Binding Protein B (S-100B) in Healthy Young Men. *Sleep*, 37(1), 195-198. doi:10.5665/sleep.3336
- Berti, V., Pupi, A., & Mosconi, L. (2011). PET/CT in diagnosis of dementia. *Annals of the New York Academy of Sciences*, 1228, 81-92. doi:10.1111/j.1749-6632.2011.06015.x
- Bird, S. M., & Fairweather, C. B. (2007). Military fatality rates (by cause) in Afghanistan and Iraq: a measure of hostilities. *International Journal of Epidemiology*, 36(4), 841-846. doi:10.1093/ije/dym103
- Bochicchio, G. V., Lumpkins, K., O'connor, J., Simard, M., Schaub, S., Conway, A., . . . Scalea, T. M. (2008). Blast injury in a civilian trauma setting is associated with a delay in diagnosis of traumatic brain injury. *The American Surgeon*, 74(3), 267-270.
- Bumbaširevic, M., Lesic, A., Mitkovic, M., & Bumbaširevic, V. (2006). Treatment of Blast Injuries of the Extremity. *JAAOS - Journal of the American Academy of Orthopaedic Surgeons*, 14(10), S77-S81.

- Capehart, B., & Bass, D. (2011, 2011/07//). Traumatic brain injury among veterans returning from Afghanistan and Iraq: strategies for diagnosis and treatment. *Psychiatric Times*, 28, 30.
- Carmeliet, P. (2003). Blood vessels and nerves: common signals, pathways and diseases. *Nature Reviews Genetics*, 4, 710. doi:10.1038/nrg1158
- Chapman, R. M., Mapstone, M., McCrary, J. W., Gardner, M. N., Porsteinsson, A., Sandoval, T. C., . . . Reilly, L. A. (2011). Predicting Conversion from Mild Cognitive Impairment to Alzheimer's Disease Using Neuropsychological Tests and Multivariate Methods. *Journal of Clinical and Experimental Neuropsychology*, 33(2), 187-199. doi:10.1080/13803395.2010.499356
- Charalampopoulos, I., Alexaki, V.-I., Tsatsanis, C., Minas, V., Dermitzaki, E., Lasaridis, I., . . . Gravanis, A. (2006). Neurosteroids as Endogenous Inhibitors of Neuronal Cell Apoptosis in Aging. *Annals of the New York Academy of Sciences*, 1088(1), 139-152. doi:10.1196/annals.1366.003
- Compston, A. (1993). Inflammation and the brain. *Molecular and Chemical Neuropathology*, 19(1), 47-64. doi:10.1007/bf03160168
- Dadas, A., Washington, J., Marchi, N., & Janigro, D. (2016). Improving the clinical management of traumatic brain injury through the pharmacokinetic modeling of peripheral blood biomarkers. *Fluids and Barriers of the CNS*, 13, 21. doi:10.1186/s12987-016-0045-y
- Daniel F. Kelly, Neil A. Martin, Rouzbeh Kordestani, George Counelis, David A. Hovda, Marvin Bergsneider, . . . Donald P. Becker. (1997). Cerebral blood flow as a predictor of outcome following traumatic brain injury. *Journal of Neurosurgery*, 86(4), 633-641. doi:10.3171/jns.1997.86.4.0633
- Deller, T., Haas, C. A., Freiman, T. M., Phinney, A., Jucker, M., & Frotscher, M. (2006). Lesion-Induced Axonal Sprouting in the Central Nervous System. In M. Bähr (Ed.), *Brain Repair* (pp. 101-121). Boston, MA: Springer US.
- DePalma, R. G., Burris, D. G., Champion, H. R., & Hodgson, M. J. (2005). Current concepts: Blast injuries. *New England Journal of Medicine*, 352(13), 1335-1399. doi:10.1056/NEJMra042083
- Dinarello, C. A. (2000). Proinflammatory Cytokines. *Chest*, 118(2), 503-508. doi:10.1378/chest.118.2.503
- Duchnick, J. J., Vanderploeg, R. D., & Curtiss, G. (2002). Identifying Retrieval Problems Using the California Verbal Learning Test. *Journal of Clinical and Experimental Neuropsychology*, 24(6), 840-851. doi:10.1076/jcen.24.6.840.8405
- Esopenko, C., & Levine, B. (2015). Aging, Neurodegenerative Disease, and Traumatic Brain Injury: The Role of Neuroimaging. *Journal of Neurotrauma*, 32(4), 209-220. doi:10.1089/neu.2014.3506
- Farrer, L. A., Cupples, L., Haines, J. L., & et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein e genotype and alzheimer disease: A meta-analysis. *JAMA*, 278(16), 1349-1356. doi:10.1001/jama.1997.03550160069041
- Fleminger, S., Oliver, D., Lovestone, S., Rabe-Hesketh, S., & Giora, A. (2003). Head injury as a risk factor for Alzheimer's disease: the evidence 10 years on; a partial replication. *Journal of Neurology, Neurosurgery, and Psychiatry*, 74(7), 857-862. doi:10.1136/jnnp.74.7.857
- Flood, J. F., Morley, J. E., & Roberts, E. (1992). Memory-enhancing effects in male mice of pregnenolone and steroids metabolically derived from it. *Proceedings of the National Academy of Sciences of the United States of America*, 89(5), 1567-1571.

- Fratiglioni, L., Grut, M., Forsell, Y., Viitanen, M., Grafström, M., Holmen, K., . . . Winblad, B. (1991). Prevalence of Alzheimer's disease and other dementias in an elderly urban population Relationship with age, sex, and education. *Neurology*, *41*(12), 1886-1886.
- Fullerton, S. M., Clark, A. G., Weiss, K. M., Nickerson, D. A., Taylor, S. L., Stengård, J. H., . . . Sing, C. F. (2000). Apolipoprotein E Variation at the Sequence Haplotype Level: Implications for the Origin and Maintenance of a Major Human Polymorphism. *American Journal of Human Genetics*, *67*(4), 881-900.
- Galindo, L. T., Filippo, T. R. M., Semedo, P., Ariza, C. B., Moreira, C. M., Camara, N. O. S., & Porcionatto, M. A. (2011). Mesenchymal Stem Cell Therapy Modulates the Inflammatory Response in Experimental Traumatic Brain Injury. *Neurology Research International*, *2011*, 564089. doi:10.1155/2011/564089
- Gao, W., Li, F., Zhou, Z., Xu, X., Wu, Y., Zhou, S., . . . Zhang, J. (2017). IL-2/Anti-IL-2 Complex Attenuates Inflammation and BBB Disruption in Mice Subjected to Traumatic Brain Injury. *Frontiers in Neurology*, *8*(281). doi:10.3389/fneur.2017.00281
- Gatson, J. W., Stebbins, C., Mathews, D., Harris, T. S., Madden, C., Batjer, H., . . . Minei, J. P. (2016). Evidence of increased brain amyloid in severe TBI survivors at 1, 12, and 24 months after injury: report of 2 cases. *Journal of Neurosurgery*, *124*(6), 1646-1653. doi:10.3171/2015.6.Jns15639
- Gavett, B. E., Stern, R. A., Cantu, R. C., Nowinski, C. J., & McKee, A. C. (2010). Mild traumatic brain injury: a risk factor for neurodegeneration. *Alzheimer's Research & Therapy*, *2*(3), 18-18. doi:10.1186/alzrt42
- Goldman, S. M., Tanner, C. M., Oakes, D., Bhudhikanok, G. S., Gupta, A., & Langston, J. W. (2006). Head injury and Parkinson's disease risk in twins. *Annals of Neurology*, *60*(1), 65-72. doi:10.1002/ana.20882
- He, J., Evans, C.-O., Hoffman, S. W., Oyesiku, N. M., & Stein, D. G. (2004). Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Experimental Neurology*, *189*(2), 404-412. doi:<https://doi.org/10.1016/j.expneurol.2004.06.008>
- He, J., Hoffman, S. W., & Stein, D. G. (2004). Allopregnanolone, a progesterone metabolite, enhances behavioral recovery and decreases neuronal loss after traumatic brain injury. *Restorative Neurology and Neuroscience*, *22*(1), 19-31.
- Houlden, H., & Greenwood, R. (2006). Apolipoprotein E4 and traumatic brain injury. *Journal of Neurology, Neurosurgery, and Psychiatry*, *77*(10), 1106-1107. doi:10.1136/jnnp.2006.095513
- Huebbe, P., Jofre-Monseny, L., & Rimbach, G. (2009). Alpha-tocopherol transport in the lung is affected by the apoE genotype—studies in transgenic apoE3 and apoE4 mice. *IUBMB life*, *61*(4), 453-456.
- Iancu, I., Tchernihovsky, E., Maayan, R., Poreh, A., Dannon, P., Kotler, M., . . . Strous, R. D. (2007). Circulatory neurosteroid levels in smoking and non-smoking chronic schizophrenia patients. *European Neuropsychopharmacology*, *17*(8), 541-545. doi:<https://doi.org/10.1016/j.euroneuro.2007.02.001>
- Katzman, R. (1976). The prevalence and malignancy of alzheimer disease: A major killer. *Archives of Neurology*, *33*(4), 217-218. doi:10.1001/archneur.1976.00500040001001
- Kimbrel, N. A., Hauser, M. A., Garrett, M., Ashley-Koch, A., Liu, Y., Dennis, M. F., . . . Beckham, J. C. (2015). Effect of the APOE ε4 Allele and Combat Exposure on PTSD

- among Iraq/Afghanistan-Era Veterans. *Depression and anxiety*, 32(5), 307-315.
doi:10.1002/da.22348
- Koenig, H., Schumacher, M., Ferzaz, B., Thi, A., Ressouches, A., Guennoun, R., . . . Baulieu, E. (1995). Progesterone synthesis and myelin formation by Schwann cells. *Science*, 268(5216), 1500-1503. doi:10.1126/science.7770777
- Kristiansen, O. P., & Mandrup-Poulsen, T. (2005). Interleukin-6 and Diabetes. *The Good, the Bad, or the Indifferent?*, 54(suppl 2), S114-S124. doi:10.2337/diabetes.54.suppl_2.S114
- Lachapelle, J., Bolduc-Teasdale, J., Ptito, A., & McKerral, M. (2008). Deficits in complex visual information processing after mild TBI: Electrophysiological markers and vocational outcome prognosis. *Brain Injury*, 22(3), 265-274. doi:10.1080/02699050801938983
- Langlois, J. A., Kegler, S. R., Butler, J. A., Gotsch, K. E., Johnson, R. L., Reichard, A. A., . . . Thurman, D. J. (2003). Traumatic Brain Injury-Related Hospital Discharges Results from a 14-State Surveillance System, 1997. *Morbidity and Mortality Weekly Report: Surveillance Summaries*, 52(SS-4), 1-20.
- Laskowitz, D., & Grant, G. (2016). *Translational research in traumatic brain injury* (Vol. 57): CRC Press.
- Launer, L. J., Andersen, K., Dewey, M. E., Letenneur, L., Ott, A., Amaducci, L. A., . . . Hofman, A. (1999). Rates and risk factors for dementia and Alzheimer's disease. *Results from EURODEM pooled analyses*, 52(1), 78-78. doi:10.1212/wnl.52.1.78
- Li, L., Bao, Y., He, S., Wang, G., Guan, Y., Ma, D., . . . Yang, J. (2015). The Association Between Apolipoprotein E and Functional Outcome After Traumatic Brain Injury: A Meta-Analysis. *Medicine*, 94(46), e2028. doi:10.1097/MD.0000000000002028
- Lowther, D. E., & Hafler, D. A. (2012). Regulatory T cells in the central nervous system. *Immunological Reviews*, 248(1), 156-169. doi:10.1111/j.1600-065X.2012.01130.x
- Lundin, A., de Bussard, C., Edman, G., & Borg, J. (2006). Symptoms and disability until 3 months after mild TBI. *Brain Injury*, 20(8), 799-806.
- Lynch, J. R., Tang, W., Wang, H., Vitek, M. P., Bennett, E. R., Sullivan, P. M., . . . Laskowitz, D. T. (2003). APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *Journal of Biological Chemistry*, 278(49), 48529-48533.
- Maggio, M., Guralnik, J. M., Longo, D. L., & Ferrucci, L. (2006). Interleukin-6 in Aging and Chronic Disease: A Magnificent Pathway. *The journals of gerontology. Series A, Biological sciences and medical sciences*, 61(6), 575-584.
- Mahley, R. W., & Stanley C. Rall, J. (2000). Apolipoprotein E: Far More Than a Lipid Transport Protein. *Annual Review of Genomics and Human Genetics*, 1(1), 507-537.
doi:10.1146/annurev.genom.1.1.507
- Martin, E. M., Lu, W. C., Helmick, K., French, L., & Warden, D. L. (2008). Traumatic Brain Injuries Sustained in the Afghanistan and Iraq Wars. *AJN The American Journal of Nursing*, 108(4), 40-47. doi:10.1097/01.NAJ.0000315260.92070.3f
- Marx, C. E., Trost, W. T., Shampine, L. J., Stevens, R. D., Hulette, C. M., Steffens, D. C., . . . Lieberman, J. A. (2006). The Neurosteroid Allopregnanolone Is Reduced in Prefrontal Cortex in Alzheimer's Disease. *Biological Psychiatry*, 60(12), 1287-1294.
doi:<https://doi.org/10.1016/j.biopsych.2006.06.017>
- Mathias, J. L., Beall, J. A., & Bigler, E. D. (2004). Neuropsychological and information processing deficits following mild traumatic brain injury. *Journal of the International Neuropsychological Society*, 10(2), 286-297. doi:10.1017/S1355617704102117

- Ming, G.-l., & Song, H. (2011). Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions. *Neuron*, 70(4), 687-702. doi:10.1016/j.neuron.2011.05.001
- Monteleone, P., Luisi, M., De Filippis, G., Colurcio, B., Monteleone, P., Genazzani, A. R., & Maj, M. (2003). Circulating levels of neuroactive steroids in patients with binge eating disorder: A comparison with nonobese healthy controls and non-binge eating obese subjects. *International Journal of Eating Disorders*, 34(4), 432-440. doi:10.1002/eat.10199
- Morgan, R., Kreipke, C. W., Roberts, G., Bagchi, M., & Rafols, J. A. (2007). Neovascularization following traumatic brain injury: possible evidence for both angiogenesis and vasculogenesis. *Neurological Research*, 29(4), 375-381. doi:10.1179/016164107X204693
- Morganti-Kossmann, M. C., Rancan, M., Otto, V. I., Stahel, P. F., & Kossmann, T. (2001). Role of cerebral inflammation after traumatic brain injury: A revisited concept. *Shock*, 16(3), 165-177. doi:10.1097/00024382-200116030-00001
- Morganti-Kossmann, M. C., Satgunaseelan, L., Bye, N., & Kossmann, T. (2007). Modulation of immune response by head injury. *Injury*, 38(12), 1392-1400. doi:<https://doi.org/10.1016/j.injury.2007.10.005>
- Naylor, J. C., Kilts, J. D., Hulette, C. M., Steffens, D. C., Blazer, D. G., Ervin, J. F., . . . Marx, C. E. (2010). Allopregnanolone Levels are Reduced in Temporal Cortex in Patients with Alzheimer's Disease Compared to Cognitively Intact Control Subjects. *Biochimica et biophysica acta*, 1801(8), 951-959. doi:10.1016/j.bbali.2010.05.006
- Naylor, J. C., Kilts, J. D., Szabo, S. T., Dunn, C. E., Keefe, F. J., Tupler, L. A., . . . Hamer, R. M. (2016). Allopregnanolone levels are inversely associated with self-reported pain symptoms in US Iraq and Afghanistan-era veterans: Implications for biomarkers and therapeutics. *Pain Medicine*, 17(1), 25-32.
- Niesman, I. R., Schilling, J. M., Shapiro, L. A., Kellerhals, S. E., Bonds, J. A., Kleschevnikov, A. M., . . . Head, B. P. (2014). Traumatic brain injury enhances neuroinflammation and lesion volume in caveolin deficient mice. *Journal of Neuroinflammation*, 11, 39-39. doi:10.1186/1742-2094-11-39
- Olsen, R. H. J., Agam, M., Davis, M. J., & Raber, J. (2012). ApoE Isoform-Dependent Deficits in Extinction of Contextual Fear Conditioning. *Genes, brain, and behavior*, 11(7), 806-812. doi:10.1111/j.1601-183X.2012.00833.x
- Perneckzy, R., Diehl-Schmid, J., Pohl, C., Drzezga, A., & Kurz, A. (2007). Non-fluent progressive aphasia: Cerebral metabolic patterns and brain reserve. *Brain Research*, 1133, 178-185. doi:<https://doi.org/10.1016/j.brainres.2006.11.054>
- Pinna, G., & Rasmusson, A. M. (2012). Upregulation of neurosteroid biosynthesis as a pharmacological strategy to improve behavioral deficits in a putative mouse model of PTSD. *Journal of neuroendocrinology*, 24(1), 102-116. doi:10.1111/j.1365-2826.2011.02234.x
- Prencipe, M., Casini, A. R., Ferretti, C., Lattanzio, M. T., Fiorelli, M., & Culasso, F. (1996). Prevalence of dementia in an elderly rural population: effects of age, sex, and education. *Journal of Neurology, Neurosurgery, and Psychiatry*, 60(6), 628-633.
- Price, L., Wilson, C., & Grant, G. (2016). Blood-brain barrier pathophysiology following traumatic brain injury.

- Rasmusson, A. M., Pinna, G., Paliwal, P., Weisman, D., Gottschalk, C., Charney, D., . . . Guidotti, A. (2006). Decreased Cerebrospinal Fluid Allopregnanolone Levels in Women with Posttraumatic Stress Disorder. *Biological Psychiatry*, *60*(7), 704-713. doi:<https://doi.org/10.1016/j.biopsych.2006.03.026>
- Reverte, I., Klein, A. B., Ratner, C., Domingo, J. L., & Colomina, M. T. (2012). Behavioral phenotype and BDNF differences related to apoE isoforms and sex in young transgenic mice. *Experimental Neurology*, *237*(1), 116-125. doi:<https://doi.org/10.1016/j.expneurol.2012.06.015>
- Robertson, C. L., Fidan, E., Stanley, R. M., Mhsa, Noje, C., & Bayir, H. (2015). Progesterone for Neuroprotection in Pediatric Traumatic Brain Injury. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*, *16*(3), 236-244. doi:10.1097/PCC.0000000000000323
- Schmidt, M., Sachse, C., Richter, W., Xu, C., Fändrich, M., & Grigorieff, N. (2009). Comparison of Alzheimer A β (1–40) and A β (1–42) amyloid fibrils reveals similar protofilament structures. *Proceedings of the National Academy of Sciences*, *106*(47), 19813-19818. doi:10.1073/pnas.0905007106
- Schneiderman, A. I., Braver, E. R., & Kang, H. K. (2008). Understanding Sequelae of Injury Mechanisms and Mild Traumatic Brain Injury Incurred during the Conflicts in Iraq and Afghanistan: Persistent Postconcussive Symptoms and Posttraumatic Stress Disorder. *American journal of epidemiology*, *167*(12), 1446-1452. doi:10.1093/aje/kwn068
- Schraegle, W. A., Nussbaum, N. L., & Stefanatos, A. K. (2016). List-learning and verbal memory profiles in childhood epilepsy syndromes. *Epilepsy & Behavior*, *62*, 159-165. doi:<https://doi.org/10.1016/j.yebeh.2016.07.021>
- Silver, J., & Miller, J. H. (2004). Regeneration beyond the glial scar. *Nature Reviews Neuroscience*, *5*, 146. doi:10.1038/nrn1326
- Sivanandam, T. M., & Thakur, M. K. (2012). Traumatic brain injury: A risk factor for Alzheimer's disease. *Neuroscience & Biobehavioral Reviews*, *36*(5), 1376-1381. doi:<https://doi.org/10.1016/j.neubiorev.2012.02.013>
- Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in neurosciences*, *32*(12), 638-647. doi:10.1016/j.tins.2009.08.002
- Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta Neuropathologica*, *119*(1), 7-35. doi:10.1007/s00401-009-0619-8
- Sperry, J. L., Friese, R. S., Frankel, H. L., West, M. A., Cuschieri, J., Moore, E. E., . . . Investigators, t. H. R. t. I. (2008). Male Gender is Associated With Excessive IL-6 Expression Following Severe Injury. *Journal of Trauma and Acute Care Surgery*, *64*(3), 572-579. doi:10.1097/TA.0b013e3181650fdf
- Sripada, R. K., Marx, C. E., King, A. P., Rampton, J. C., Ho, S. S., & Liberzon, I. (2013). Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits. *Biological Psychiatry*, *73*(11), 1045-1053.
- Stroop, J. R. (1935). Studies of interference in serial verbal reactions. *Journal of experimental psychology*, *18*(6), 643.
- Stuss, D. T. (2011). Traumatic brain injury: relation to executive dysfunction and the frontal lobes. *Current opinion in neurology*, *24*(6), 584-589.

- Tanielian, T., Haycox, L. H., Schell, T. L., Marshall, G. N., Burnam, M. A., Eibner, C., . . . Vaiana, M. E. (2008). *Invisible wounds of war. Summary and recommendations for addressing psychological and cognitive injuries*. Retrieved from
- Tomanek, R. J. (2012). *Assembly of the vasculature and its regulation*: Springer Science & Business Media.
- Vagnerova, K., Koerner, I. P., & Hurn, P. D. (2008). Gender and the Injured Brain. *Anesthesia and analgesia*, 107(1), 201-214. doi:10.1213/ane.0b013e31817326a5
- Van Den Heuvel, C., Thornton, E., & Vink, R. (2007). Traumatic brain injury and Alzheimer's disease: a review. In J. T. Weber & A. I. R. Maas (Eds.), *Progress in Brain Research* (Vol. 161, pp. 303-316): Elsevier.
- VanLandingham, J. W., Cekic, M., Cutler, S., Hoffman, S. W., & Stein, D. G. (2007). Neurosteroids reduce inflammation after TBI through CD55 induction. *Neuroscience Letters*, 425(2), 94-98. doi:10.1016/j.neulet.2007.08.045
- Vergheze, P. B., Castellano, J. M., & Holtzman, D. M. (2011). Roles of Apolipoprotein E in Alzheimer's Disease and Other Neurological Disorders. *Lancet neurology*, 10(3), 241-252. doi:10.1016/S1474-4422(10)70325-2
- Wang, J. M., Johnston, P. B., Ball, B. G., & Brinton, R. D. (2005). The Neurosteroid Allopregnanolone Promotes Proliferation of Rodent and Human Neural Progenitor Cells and Regulates Cell-Cycle Gene and Protein Expression. *The Journal of Neuroscience*, 25(19), 4706-4718. doi:10.1523/jneurosci.4520-04.2005
- Wechsler, D., Coalson, D. L., & Raiford, S. E. (1997). *WAIS-III: Wechsler adult intelligence scale*: Psychological Corporation San Antonio, TX.
- Wei, J., & Xiao, G.-m. (2013). The neuroprotective effects of progesterone on traumatic brain injury: current status and future prospects. *Acta Pharmacologica Sinica*, 34(12), 1485.
- Xilouri, M., & Papazafiri, P. (2006). Anti-apoptotic effects of allopregnanolone on P19 neurons. *European Journal of Neuroscience*, 23(1), 43-54. doi:10.1111/j.1460-9568.2005.04548.x
- Xu, X., Li, M., & Jiang, Y. (2013). The Paradox Role of Regulatory T Cells in Ischemic Stroke. *The Scientific World Journal*, 2013, 174373. doi:10.1155/2013/174373
- Yi, J.-H., & Hazell, A. S. (2006). Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. *Neurochemistry International*, 48(5), 394-403. doi:<https://doi.org/10.1016/j.neuint.2005.12.001>
- Yin, M., Zhang, L., Sun, X.-m., Mao, L.-f., & Pan, J. (2010). Lack of apoE causes alteration of cytokines expression in young mice liver. *Molecular Biology Reports*, 37(4), 2049-2054. doi:<http://dx.doi.org/10.1007/s11033-009-9660-x>
- Zhu, T. S., & Glaser, M. (2008). Regulatory role of cytochrome P450scc and pregnenolone in myelination by rat Schwann cells. *Molecular and Cellular Biochemistry*, 313(1), 79-89. doi:10.1007/s11010-008-9745-1

Table 1. Demographics of Longitudinal Sample

Race	Age	TBI status	Year of Neurocognitive Baseline Measures
African American	54	y	2006
Caucasian	46	y	2007
Caucasian	54	y	2007
Caucasian	38	n	2007
Caucasian	49	n	2007
African American	46	y	2007
African American	49	y	2007
African American	55	y	2008
Caucasian	56	y	2008
Caucasian	42	n	2008

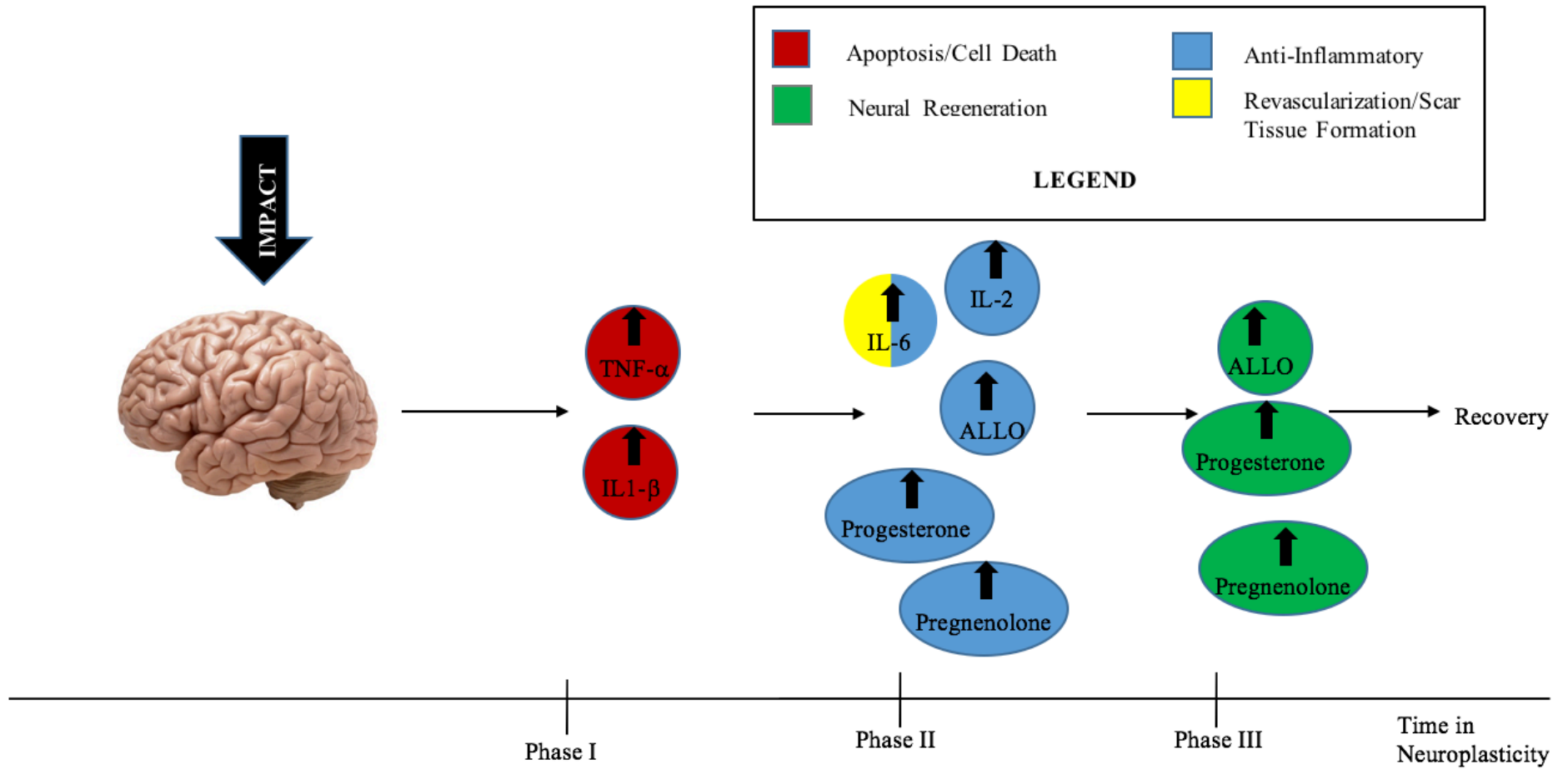


Figure 1. This tripartite mechanism of recovery is displayed pictographically post injury to the brain

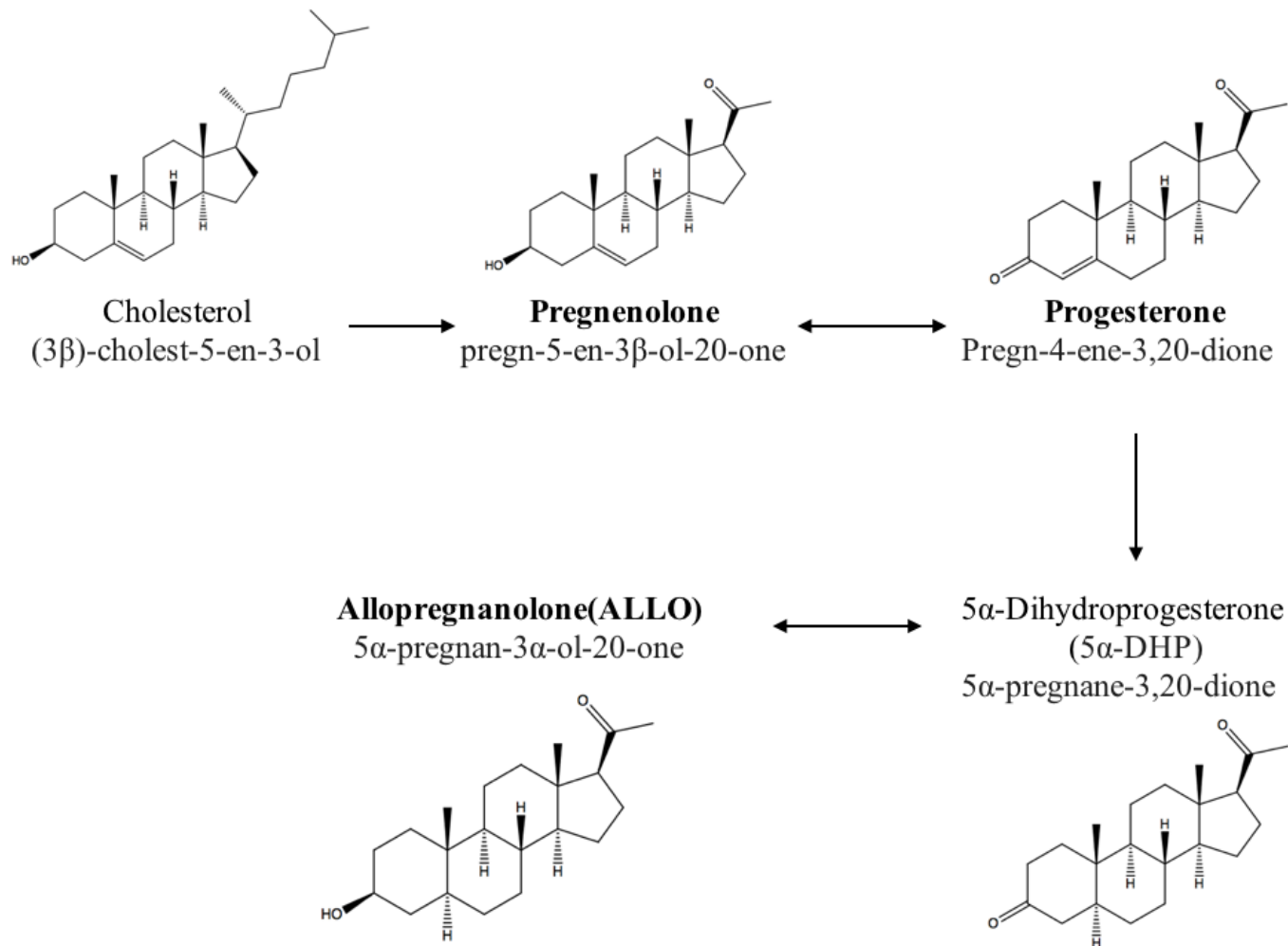


Figure 2. A schematic of the catabolism of the neurosteroids thought to be most relevant to neuroplasticity are presented

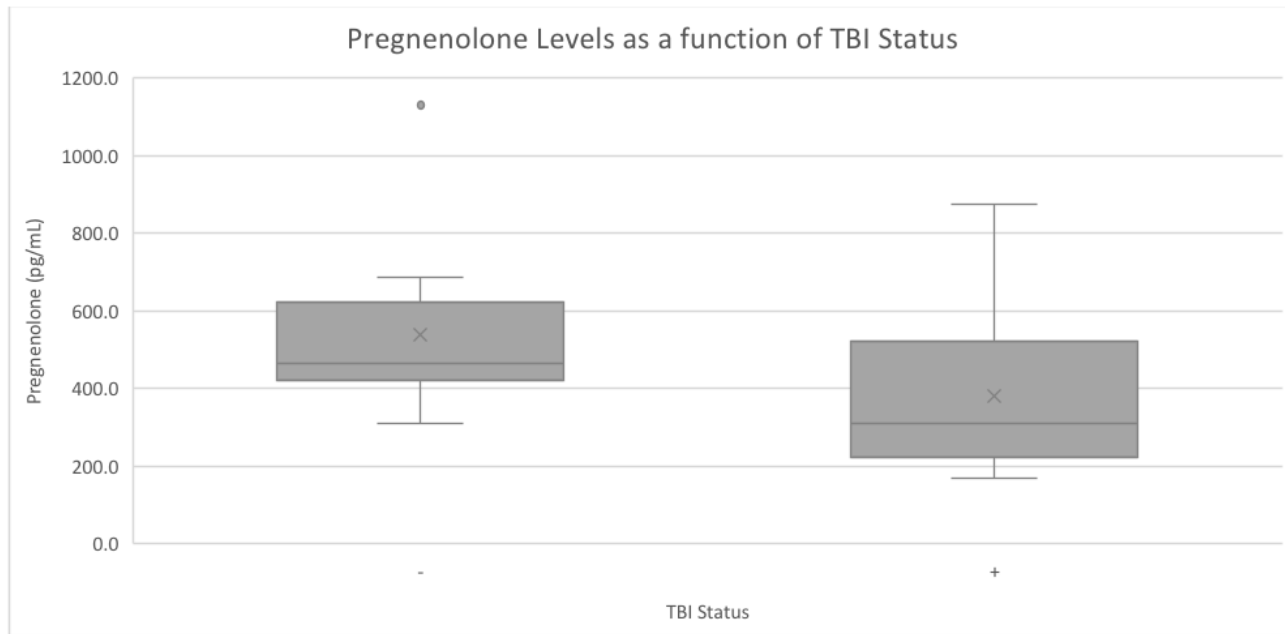


Figure 3. Significant differences in pregnenolone levels in those with history of TBI versus those without TBI ($p=0.0438$). Participants with TBI had significantly lower values (380.6 ± 201.2 pg/mL) than those without TBI (539.2 ± 207.3 pg/mL).

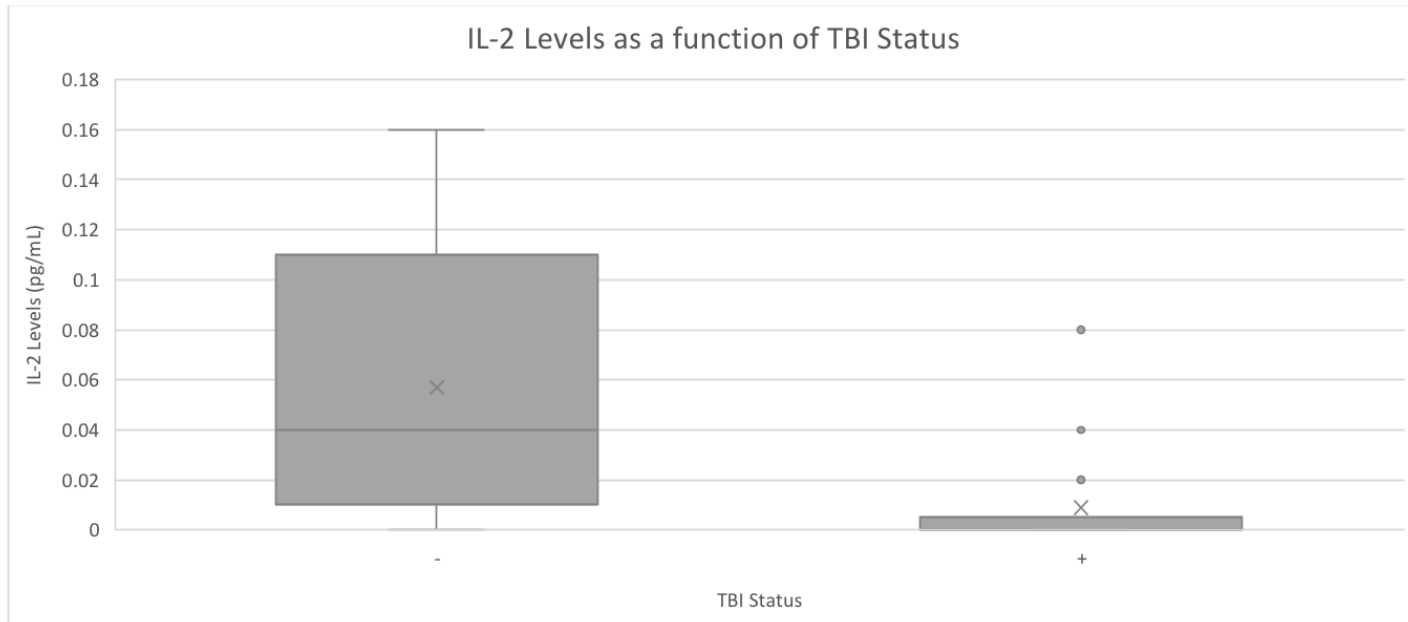


Figure 4. Significant differences in IL-2 levels in those with history of TBI versus those without TBI ($p=0.0021$). Participants with TBI had a significantly lower mean amount of IL-2 (0.009 ± 0.02 pg/mL) compared with those without TBI (0.06 ± 0.06 pg/mL).

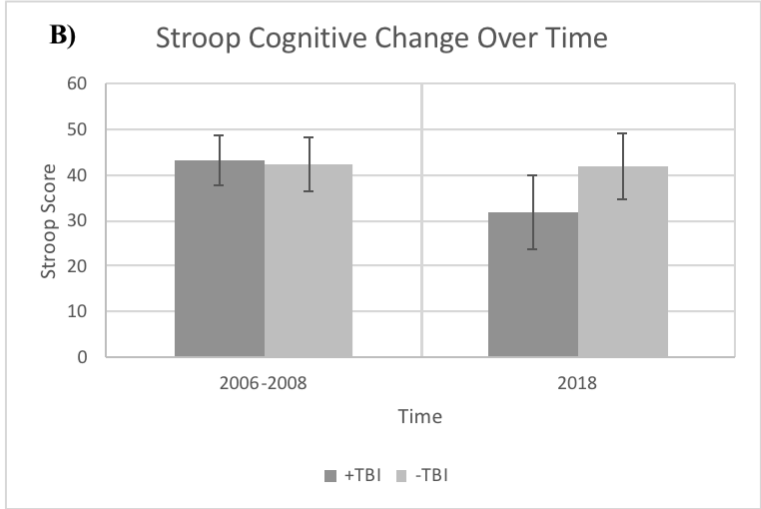
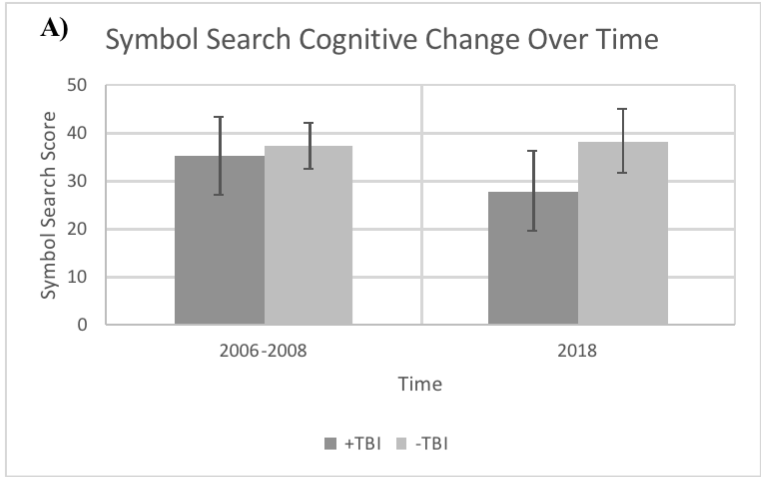


Figure 5. The significant cognitive decline in the last decade between TBI positive and TBI negative subjects for the attentional tests ($p=0.0166$) for both A) Symbol Search and B) Stroop