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THE GENETIC INFLUENCE OF THE BDNF VAL66MET POLYMORPHISM ON HIPPOCAMPAL VOLUME AND MEMORY IN TRAUMATIC BRAIN INJURY SUBJECTS

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ABSTRACT

Given the ever-growing number of traumatic brain injury cases every year, the research surrounding the recovery and outcome of subjects has both greatly expanded and advanced. MRI T1-weighted images are used to determine the anatomical deficits caused by the injuries and the brain regions most affected. The hippocampus atrophies following an injury include decreased volume and diminished memory, attention, and cognitive functions. In previous neuroscience research, genetic variations have been observed to influence the recovery and outcome of injury patients. Despite the research surrounding genetics and injuries, few studies have been conducted to examine the genetic risk factors of the BDNF alleles predicting the hippocampal volume. The current study examines the effects of the val66met BDNF polymorphism on hippocampal volume and atrophy of traumatic brain injury subjects. Memory and attention deficits of TBI subjects positive for the BDNF polymorphism and TBI subjects negative for the polymorphism were also assessed through the Visual Search and Attention Test and the Digit Span Forward and Backwards Test. Findings reveal that the injury severity of TBI subjects interacts with the BDNF genetic predisposition to predict hippocampus volume. Given a larger sample size, the presence of the val66met BDNF polymorphism may be used to significantly predict hippocampal atrophy following a traumatic brain injury.

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Introduction

There are 1.7 million cases of traumatic brain injuries (TBI) in the United States each year. Approximately 52,000 of these injuries result in death annually (Centers for Disease Control and Prevention [CDC], 2016). The TBI rate of incidence has been increasing over the past few decades. Moreover, TBIs are the leading causes of injury and death of young people. The leading causes of TBI are falls, sports injuries, and motor vehicle accidents. TBIs are most common in young people, with the majority occurring in males (75%), and the elderly. A TBI is the result of external forces coming into contact with the head and causing damage. During initial admittance to a hospital following injury, trained medical professionals use the Glasgow Coma Scale (GCS) to describe the presenting patient's level of consciousness. The Scale tests for eye movement and attentiveness, verbal responses, and motor responses (Jones, 1979). Patients with higher GCS scores are more conscious and have more functional outcomes during and following the recovery process (Zafonte et al., 1996; Katz et al., 1994).

With improvements in technologies regarding brain-imaging methods (MRI, fMRI, CT, DTI) over the last few decades, an increasing number of TBI cases are diagnosed and successfully treated (McAllister et al., 1999; Jiang, et al., 2011; Lee et al., 2008; Laatsch, 2007; Xu et al., 2007). In recent years, research has begun to examine the relationship between volumes of various brain regions and brain region function finding that specific cognitive functions worsen as the volume of certain brain regions decrease (Tate and Bigler, 2000; Cohen et al., 2007; Warner et al., 2010; Trivedi et al., 2007). Cognitive function changes include

deficits in memory, learning, processing speed, and executive functioning (Trivedi et al., 2007; Colcombe et al., 2006).

MRI brain imaging methods are used to determine regional brain volume differences affected by diseases and injuries such as TBI (Tate and Bigler, 2000), Alzeheimer's Disease (Peterson et al., 2000), Multiple Sclerosis (Gasperini et al., 2002), and Schizophrenia (Steen et al., 2006). Memory and attention deficits have been studied in the recovery process of many TBI subjects. Many of these deficits are related to injuries and resulting changes within the hippocampus (Laroche et al., 2000; Hariri et al., 2003; Egan et al., 2003). The hippocampus is just one of the many brain regions involved in human memory and attention. It is, however, one of the most researched brain regions involving working, episodic, and spatial memory because of its drastic impact on memory related cognitive functioning (Laroche et al., 2007; Burgess et al., 2002). Neuropsychology tests, such as visual search and attention tasks and the Digit Span Tests Forward and Backwards, are used to measure such cognitive functioning, specifically the levels of attention and memory (Giesbrecht et al., 2013; Cave, 1992).

In recent years, genetics have gained traction in predicting and describing the recovery process following TBIs and there are many different processes through which genes have impacted brain regions following TBIs (Napieralski et al., 1999; Iadecola et al., 1997). Certain genes, such as ApoE and BDNF, have proven to be predictive of TBI recovery and post-injury executive functioning (Lichtman et al., 2000; Kruger et al., 2011).

Hippocampus and Changes in Volume

The hippocampus, along with the cingulate cortex, olfactory cortex and amygdala, are brain structures of the limbic system. The hippocampus is a particularly important brain region for the formation and retention of memories (Eichenbaum and Fortin, 2005; Squire, 1992). The hippocampus interacts with many other regions of the brain, such as the hypothalamus, fornix, and the prefrontal cortex to communicate, control stress, and consolidate memories (Phillips et al., 2006; Preston et al., 2013). The hippocampus is best seen in the coronal view of an MRI, yet is not present in many of the image slides (Duvernoy et al, 2005).

Both the right and left hippocampi play different roles regarding memories, dependent on both the type and the nature of the memories (Squire et al., 1992). Left hippocampal impairments are associated with verbal memory deficits (Frisk & Milner, 1990) while right hippocampal impairments are associated with spatial memory deficits (Smith & Milner, 1989). Decreases in volume of the specific hemispheres will result in the particular deficits dependent on the area of injury or disease.

In previous studies, decreases in hippocampal volume have been linked to depression (Bremner et al., 2000), post-traumatic stress disorder (Bremner et al., 1995), and Alzheimer's Disease (Schuff et al., 2009). Hippocampal atrophy is also predictive of decreased memory function in TBI subjects (Peterson et al., 2000; Bigler et al., 1997; Tate & Bigler, 2000).

BDNF's Genetic Role in Impact

Many genes have been successfully linked to an influence of neuronal plasticity. Brain-derived neurotrophic factor (BDNF) is a neurotrophin, which aids in neuronal survival and

neurogenesis (Failla, 2015). The BDNF gene also influences the neurotrophin factor BDNF. The BDNF gene has a polymorphism resulting in an amino acid substitution of valine (Val) to methionine (Met) at codon 66 (val66met). Roughly 30% of the population is positive for the val66met polymorphism. The Met allele is associated with abnormal hippocampal activation (Egan et al., 2003). The polymorphism affects the intracellular tracking and secretion of BDNF and the BDNF gene consequently plays a role in human memory through the facilitation of long-term potentiation (Egan et al., 2003).

Hippocampal neurogenesis can be altered by genetic influences (Sahay et al., 2011). Certain genetic predispositions, for example, alter the amount of neurogenesis that occurs in the hippocampus of adult brains (Kempermann & Gage, 2002). Neurogenesis in the hippocampus can occur after TBI to recover function lost due to the damage induced by the injuries (Rola et al., 2006). Genetic regulation through genes such as FGF-2 can alter the amount of neurogenesis following a TBI (Yoshimura et al., 2001). Genes play a major role in how the hippocampus functions. With the addition of a TBI event, the genetic contribution increases drastically throughout the recovery process.

It also has to be taken into consideration, however, that despite the analysis of the effect of the BDNF gene, TBI subjects still experience a decrease in their hippocampal volume due to atrophy post injury (Bigler 1997). To be certain, the severity of the injury determines the gravity of the outcome and also predicts the degree of memory impairment. However, the hippocampal atrophy is not the sole cause of the change in memory (Tate and Bigler 2000). Met-BDNF alleles are also seen to have 11% smaller hippocampal volumes when compared to the homozygous val population (Bueller 2006).

The Met allele, even in healthy controls, has affected both working memory and hippocampal functioning (Hariri et al., 2003) while TBI subjects also show a decrease in hippocampal volume (Bigler, 1997). Together, the decreases in volume and hippocampal atrophy might be even more severe after injury, affecting the recovery and memory function of TBI subjects with the BDNF polymorphism.

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) has been used since the 1980s in many clinical applications to diagnose injuries and disorders of the brain and many other areas of the body. MRIs are most useful in areas of the brain without bone and, unlike CT scans, do not expose the subject to harmful levels of radiation.

In an MRI of the brain, a very powerful magnet aligns the protons of the water in the brain to the field of the magnet. A radio wave is then sent to knock the protons out of alignment with the magnet. The protons spin out of equilibrium. Once the current is gone, the time is measured to determine how long it takes for the protons to realign with the magnet to achieve equilibrium. The measured time is dependent on both the nature of the protons and the environment in which they are located. The differences in times are detailed in the images created. T1 images are used to differentiate anatomical structures along the parameters of short Repetition Time (TR) and short Echo Time (TE). The contrast in T1 image intensities is dependent on the differences in proton longitudinal magnetization recovery. In T1-weighted images, the cerebrospinal fluid and other fluids appear dark. Therefore, the ventricles appear

dark in a T1 image. Gray matter is also darker than white matter and such contrasts allow analysis (Berger, 2002).

Magnetic resonance produces very precise images that can be used to differentiate the various structures of the brain, such as the hippocampus. Through the mapping of the hippocampus, its volume can be measured for comparison between healthy controls and TBI subjects.

Goals of the Current Study

The presence of the BDNF genetic polymorphism can be observed to determine if it plays a role in advanced hippocampal atrophy following a TBI. When looking at BDNF and GCS, and other measures for TBI outcomes, the hippocampal volume of TBI subjects might be predictive. The total volume of the hippocampi might also be predictive of the subjects' scores on certain neuropsychology testing for both memory and attention.

Current Study

Hypothesis 1: TBI subjects will have smaller hippocampi than the hippocampi of the HC subjects.

Hypothesis 2: TBI subjects who are positive for val66met will have smaller hippocampal volumes than TBI subjects who are negative for val66met.

Hypothesis 3: TBI subjects who are positive for val66met will have lower (worse) scores on Cognitive Tests when compared to the TBI subjects who are negative for val66met

Hypothesis 4: Injury severity of TBI subjects will interact with genetic predisposition to predict hippocampus volume

Methods

Subjects

The study involved 40 individuals between the ages of 18 and 79, including 21 with moderate to severe TBIs and 19 healthy adults of comparable age and education (see Table 1). The participants were divided into four groups: healthy control (HC) with the BNDF Val66Met polymorphism (HC positive), healthy control without Val66Met polymorphism (HC negative), TBI with val66met polymorphism (TBI positive), and TBI without val66met polymorphism (TBI negative). The subjects were matched for age and education.

Table 1. Subject Demographic means and standard deviations for healthy control and Traumatic Brain Injury groups

	HC (Mean, SD)	TBI (Mean, SD)
	N=19	N=21
Gender	11 M, 8 F	13 M, 8 F
Age (years)	37.68, 15.463	30.29, 12.87
Education (years)	12.395, 1.658	12.80, 1.67
Acute Stay in Hospital (days)	N/A	14.69, 10.21
Injury Severity GCS	N/A	5.60, 3.393

All of the TBI subjects were diagnosed with a TBI based on the CDC definition of a TBI which reads, “damage to brain tissue causes by an external mechanical force, as evidenced by

loss of consciousness due to brain trauma, post-traumatic amnesia, skull fracture, or objective neurological findings that can be reasonably attributed to TBI on physical examination or mental status examination." The Glasgow Coma Scale (GCS) was used to define severity of TBIs in the first 24 hours after injury as a measure of consciousness (Teasdale, 1974). A GSC score of 3-8 was considered "severe" and a GSC score of 9-12 was considered "moderate". The number of days the subjects stayed in the hospital post injury was also recorded.

Genetics Sequencing

To determine the presence of the BDNF val66met polymorphism, genetic sequencing was run and individuals were assigned to either a positive or negative val66met polymorphism group. DNA Extraction and genotyping was performed at the Genomics Core Facility in the Huck Institute of the Life Sciences at Penn State University under the direction of Deborah S. Grove, Director for Genetic Analysis. DNA extraction was performed using methods and materials based on Freeman et al. (2003). Buccal mucosa cells were collected from subjects with cotton swabs and placed in 15-ml centrifuge tubes containing 2.5 mls of lysis buffer. After extraction, the DNA was resuspended in 250 ul of Tris EDTA (10 mM Tris-HCl, 1mM EDTA, pH 8.0) buffer. A Nanodrop spectrophotometer was used to quantify the DNA with absorbance at 260nm. Samples were then stored in an -80^{o}C freezer.

Following the DNA extraction, the TaqMan™ SNP BDNF Assay was performed using an allelic discrimination assay protocol (Life Technologies, Carlsbad, CA). The BDNF assay that was used is C__11592758_10 for SNP rs6265. 5 microliters of the purified DNA was combined with 2X Universal PCR Mix and 1/40 the volume of the TaqMan™ SNP BDNF

reagent in a 384 well plate. A pre-read was then performed. The thermal cycling was then completed with the following protocol: a 10 minute hold at 95°C, followed by 40 to 45 cycles of 15 seconds at 92°C and 1.5 minutes at 60°C in a QuantStudio System 12K FLEX (Life Technologies, Carlsbad, CA). A post-read was performed after the amplification. The determination of the assay was done manually and automatically based on the segregation of clusters of homozygous and heterozygous subjects. 15 subjects were positive for the val66met polymorphism, either homozygous or heterozygous. 25 subjects were negative for the polymorphism, having val66val in the homozygous manner.

Table 2. Subgroup Demographic means and standard deviations

	HC Positive val66met (mean, SD)	HC Negative val66met (mean, SD)	TBI Positive val66met (mean, SD)	TBI Negative val66met (mean, SD)
	N=5	N=14	N=10	N=11
Gender	3 M, 2 F	8 M, 6 F	6 M, 4 F	7 M, 4 F
Age (years)	40.0, 11.79	36.86, 16.90	29.30, 13.45	31.18, 12.90
Education (years)	14.3, 1.71	13.07, 1.54	12.6, 1.07	13.00, 2.16
Acute Time in Hospital (days)	N/A	N/A	16.22, 10.70	12.71, 10.00
Injury Severity GCS	N/A	N/A	5.40, 2.32	5.80, 4.34

MRI Data

Subjects were scanned using either a Philips Achieva 3T scanner in the Department of Radiology at Hershey Medical Center, Hershey, PA or in one of two Siemens Magnetom trio 3T scanners (Social, Life, and Engineering Sciences Imaging Center at the Pennsylvania State University in University Park, PA or Department of Radiology at Hershey Medical Center in Hershey, PA).

Subjects were instructed to remain as motionless as possible. A 3D high-resolution T1 weighted brain anatomical images of high resolution with isotropic spatial resolution of 1.0 mm x 1.0 mm x 1.0 mm were acquired using an MPRAGE sequence: 2000 ms/2.03 ms/9 ° (repetition time (TR)/echo time (TE)/flip angle (FA), 256 x 256 mm^2 field of view (FOV), and 256 x 256 acquisition matrix with 1 mm slices.

MRI Data Preprocessing

The DICOM file for each scan was converted for NifTi format (.nii.gz) by the program dcm2nii. The center of the brain image in fMRI of the Brain Software Library (FSL) was oriented at (0,0,0) along the x-, y-, z-axes. The T1 image was preprocessed by SPM 8 to reduce noise, remove non-brain tissue from the scan, and segment the brain into grey matter, white matter, and cerebrospinal fluid (CFS). The images were aligned and the voxels were adjusted and sliced to each be 1x1x1 mm.

Data Analysis with FSL Mapping

fMRI of the Brain Software Library (FSL) version 5.0 was used to analyze the T1 scan of the subjects. The converted file was loaded in FSL and the FIRST segmentation tool was used to model and map the right and left hippocampi. FIRST uses models from previously manually segmented images of the brain by the Center of Morphometric Analysis (CMA).

FIRST was run using the command: *run_first_all -i <name of your t1 image file> -s L_Hipp,R_Hipp \ -o <output file name>*. The "-i" was used as the command for the input T1 image, the "–s" was used as the command to specify the structures that were segmented (the left and the right hippocampus) and the "-o" was used to specify the output image file name. The command was run and the right and left hippocampi were segmented out. The T1 image was viewed in FSLview and the mapped mask of the segmented hippocampus was overlaid by adding *t1output_all_fast_firstseg.nii.gz* to the image (See Figures 1, 2, and 3)

The volumes of the two hippocampi were determined using FSL Stats. The command *fslstats t1output_all_fast_firstseg.nii.gz –l 16.5 –u 17.5 –V* was used to calculate the volume of the left mapped hippocampus mask. The command *fslstats t1output_all_fast_firstseg.nii.gz –l 52.5 –u 53.5 -V* was used to calculate the volume of the right mapped hippocampus mask. The "-l" specified the lower limit for the intensity of the hippocampus and the "-u" specified the upper limit for the intensity of the hippocampus. The "-V" was the command for the volume for the volume of the masks in millimeters cubed (mm^3). After each command was run in the FSL terminal, the volume appeared in the terminal and was recorded for each subject. The volumes were added together to get the total hippocampal volume.

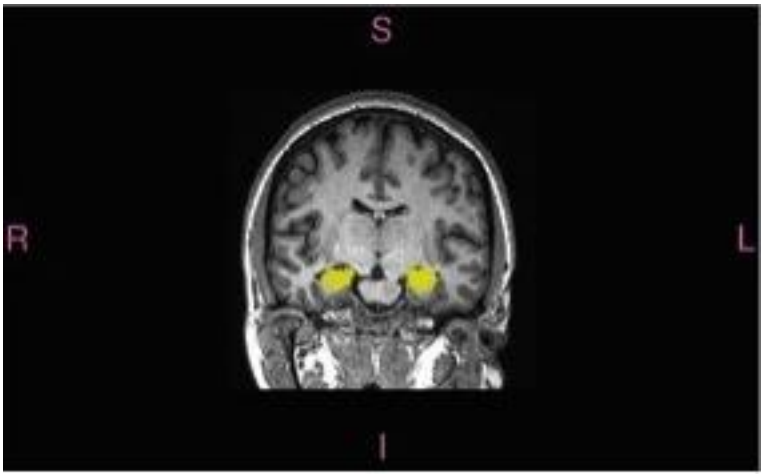

Figure 1. FSLview of TBI subject's coronal view with left and right hippocampi mapped (yellow)

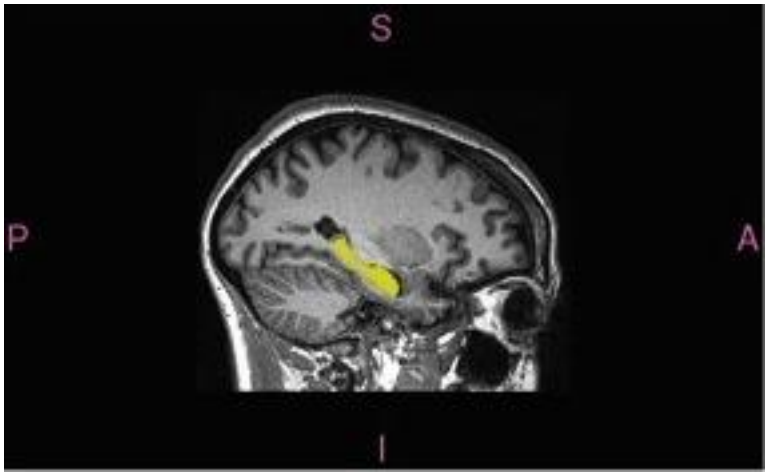

Figure 2. FLSview of TBI subject's sagittal view with hippocampi mapped (yellow)

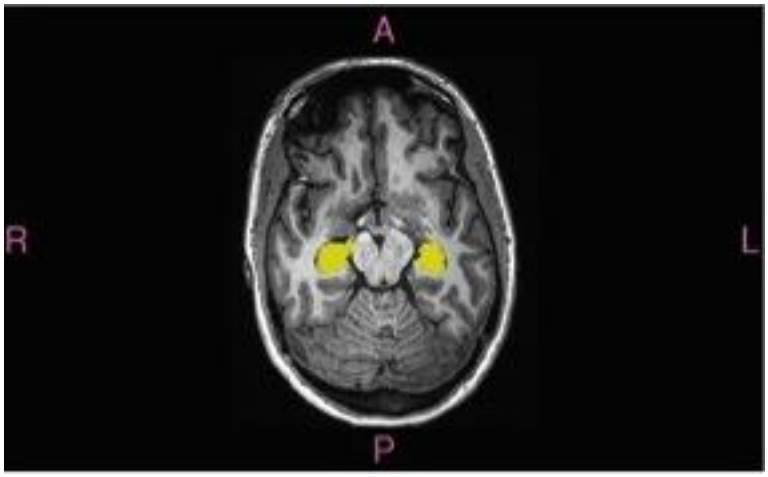

Figure 3. FSLview of TBI subject's axial view with right and left hippocampi mapped (yellow)

Neuropsychological Testing

The Digit Span of the WAIS-III (Wechsler, 1997) was administered on the day of the scan. The test was specifically targeting the working memory and attention. Both digits forward and digits backwards were administered to the subject. The length of numbers repeated back to the administrator by the subject was recorded. The forward and backwards tests were each double scored and entered to minimize recording errors. Digits Span forward was a measurement for memory attention. Digit Span backwards was a measurement for working memory. The tests were double scored and the forward and backwards tests were combined for a total score of the Digit Span Test. The Visual Search and Attention Test was also administered as a measurement for overall attention (Trenerry et al., 1990). The tests were double scored and recorded.

The hippocampal volumes and neuropsychology test scores were recorded and the means and standard deviations were calculated (Table 3).

Table 3. Total Volume and Score Means and Standard Deviations of the four subgroups

	HC Positive val66met (mean, SD)	HC Negative val66met (mean, SD)	TBI Positive val66met (mean, SD)	TBI Negative val66met (mean, SD)
Left Hippocampus Volume	3883.8, 83.4	3975.86, 630.28	3267.20, 835.72	3716.45, 380.83
Right Hippocampus Volume	3925.2, 133.76	3908, 525.57	3380.80, 1104.89	3728.91, 506.60
Total Hippocampus Volume	7809.00, 185.29	7883.85, 1114.59	6658.00, 1905.63	7505.36, 913.85
DS Forward	9.20, 1.10	10.50, 2.14	9.40, 2.413	10.27, 2.00
DS Backward	5.60, 2.30	6.07, 1.77	6.40, 1.430	7.64, 4.41
DS Total	15.40, 2.70	16.00, 3.62	15.80, 3.19	17.91, 5.83
VSAT Total	126.60, 12.20	113.29, 24.18	99.80, 20.50	113.38, 17.05

Results

Testing Hypothesis 1, an independent-sample t-test and a linear regression were conducted to compare the total hippocampal volume in traumatic brain injury subjects and healthy control subjects. There was not a significant difference in the scores for TBI subjects (Mean= 7101.86, Standard deviation= 1496.5) and healthy control subjects (Mean= 7664.16, SD= 951.84); $t(38)= -1.9$, $p= .065$. All one-sided tests were conducted with alpha of $p<0.05$. The Cohen's D effect size is d=.61. Healthy control groups have higher mean hippocampus volumes than the mean of the traumatic brain injury groups' hippocampus volumes. However, the difference is not significant.

An independent-sample t-test was conducted to compare the left hippocampal volume in traumatic brain injury subjects and healthy control subjects. There was a significant difference in the scores for TBI subjects and healthy control subjects with $p= .025$. The difference was not significant for the right hippocampal volume.

Testing Hypothesis 2, a linear regression and an independent-sample t-test were conducted to examine the total hippocampal volume in traumatic brain injury subjects positive for the val66met BDNF polymorphism and traumatic brain injury subjects negative for the val66met BDNF polymorphism. In the TBI population, the significance with which the BDNF polymorphism predicts the volume of the left hippocampus is .071. The Cohen's D effect size is d=.57. Also, BDNF had an insignificant effect on the volume size of healthy control subjects (See Figure 4).

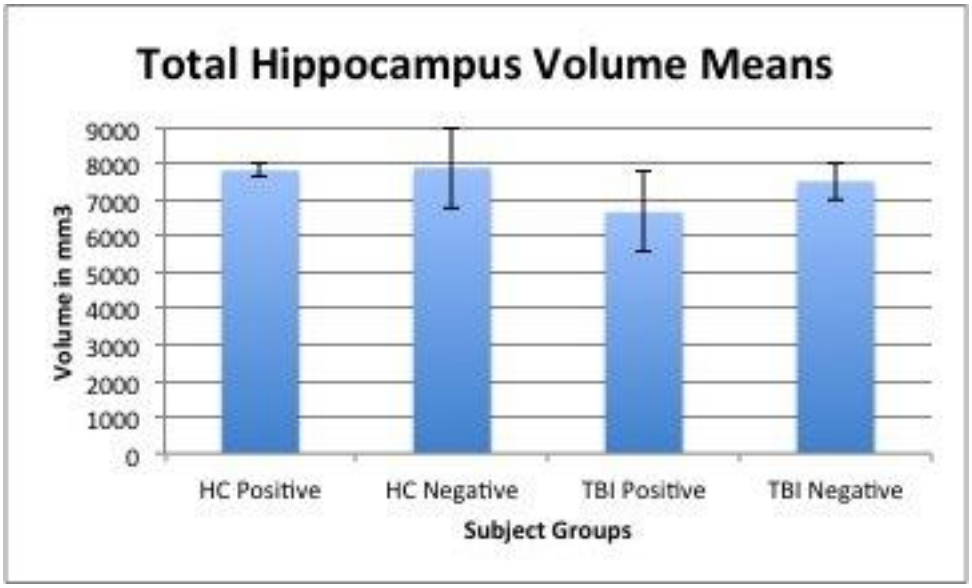

Figure 4. Total Hippocampus Volume Means for the four subgroups

Testing Hypothesis 3, an independent-sample t-test was conducted to compare the Digit Span Total (Forwards and Backwards) Mean Scores for TBI subjects positive for the val66met BDNF polymorphism and TBI subjects negative for the val66met BDNF polymorphism. There was not a significant difference in the scores for positive val66meet subjects (Mean= 15.8, Standard deviation= 3.19) and negative val66met subjects (Mean= 17.9, SD= 5.83) (See Figure 5); $t(19)= -1.01$, $p= .324$. This a not significant using the p-value, $p< .05$.

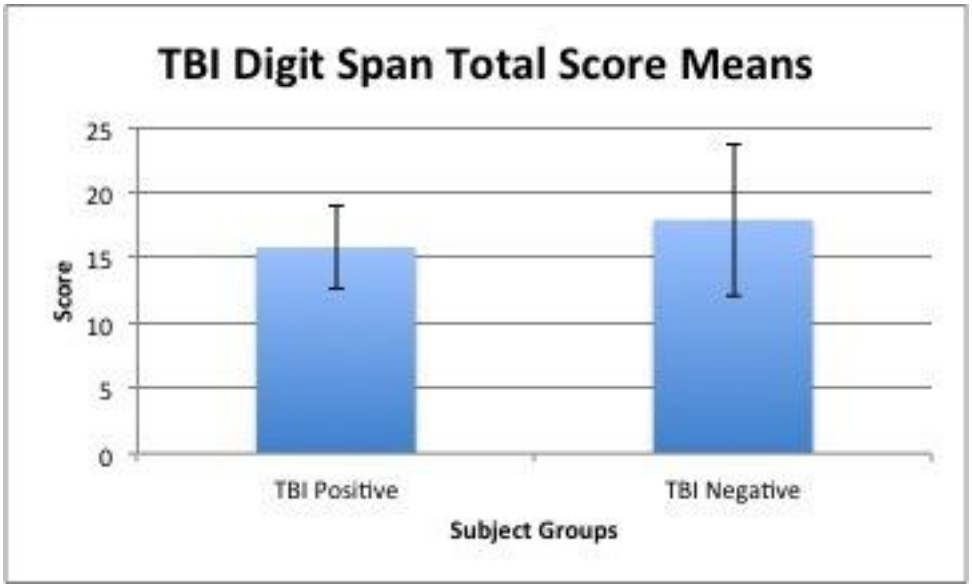

Figure 5. Comparing the Digit Span Total Score Means of BDNF polymorphism Positive and Negative TBI Groups

An independent-sample t-test was conducted to compare the Visual Search and Attention Test Mean Scores for TBI subjects positive for the val66met BDNF polymorphism and TBI subjects negative for the val66met BDNF polymorphism. There was not a significant difference in the scores for positive val66meet subjects (Mean=99.8, Standard deviation= 20.5) and negative val66met subjects (Mean= 113.4, SD= 17.1) (See Figure 6); t(12)= -1.30, p= .222. This a not significant. Using Cohen's D, the effect size is d=.45.

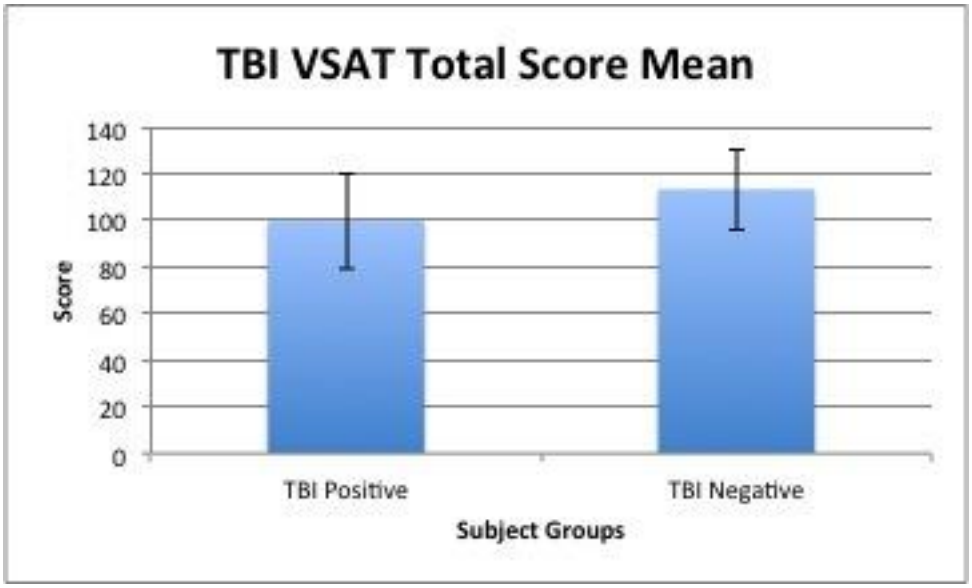

Figure 6. Comparing the VSAT Total Means of BDNF polymorphism Positive and Negative TBI Groups

Testing Hypothesis 4, a linear regression with gender and the interaction between the BDNF polymorphism and the GCS score was performed and found that using GCS as a moderator, the interaction between the presence of the val66met BDNF polymorphism and the score on the GCS scale from original admittance at the hospital significantly predicts the total volume of the hippocampus of TBI subjects; $t(19)=-2.25$, $p=.038$. Using Cohen's D, the effect size is .72.

Discussion

Primary Findings

The goal of this study was to determine if the presence of the val66met BDNF polymorphism affects the hippocampal volume and specific cognitive functions of subjects following a traumatic brain injury. The main hypothesis of this study was that the presence of the val66met polymorphism in TBI patients would result in a decrease in hippocampus volume (Egan et al, 2003). The primary findings weakly supported the hypothesis that brain injury combined with genetic risk may result in the smallest total hippocampal volume mean when compared to the other three groups' mean total hippocampus volumes. While the differences between the groups were statistically non-significant, the effect size was moderate, indicating possibly important effects to consider even in this small sample size.

Results also support previous research suggesting that hippocampal volumes for TBI subjects are smaller than the hippocampal volumes of the healthy control groups (Bigler et al., 2007). The left hippocampus volume was strongly predicted by subject's diagnosis of a traumatic brain injury. However, the decrease in left hippocampal volume did not result in worse neuropsychology test scores for the TBI subjects. Knowing that the left hippocampus is important for verbal memory deficits, decreases in the volume would have been expected to result in worse scores for the TBI group when compared to the healthy control group (Frisk & Milner, 1990).

The total hippocampal volume, while not significantly predicted by TBI diagnosis, indicated an underlying relationship with the presence of the BDNF val66met polymorphism

when compared to the TBI group negative for the polymorphism. There was a stepwise progression in hippocampal loss between Negative HC, to Positive HC, to Negative TBI and to Positive TBI.

The presence of genetic risk factors, such as BDNF and 5-HTTLPR, impact both brain volume and brain function (Pezawas et al., 2005; Mauch et al., 2001; Szeszko et al., 2005). The BDNF val66met polymorphism has been studied to reduce brain volume in both healthy control subjects and subjects with disorders such as schizophrenia and depression (Bueller, et al., 2006; Szeszko et al., 2006). Subjects with the val66met polymorphism breaks down the BDNF neurotrophins that lead to neurogenesis (Egan et al., 2003). Compared to subjects negative for the val66met polymorphism, subjects positive for the polymorphism will not have the same level of neurogenesis, which is needed, after injury, to aid in both physical and functional recovery (Roas et al., 2006).

Based on previous research supporting the notion that the BDNF val66met polymorphism results in memory impairments and that TBI subjects have decreased memory and attention, it was also predicted that TBI subjects positive for the polymorphism would perform worse on neuropsychology tests regarding the specific cognitive functions (Hariri et al, 2003; McAllister et al., 1999; Dockree et al, 2006). Our findings did not strongly support this hypothesis. Neither the VSAT nor the Digit Span Tests could be predicted in this study by the occurrence of the polymorphism.

Another primary finding of this study was the support for the hypothesis that the injury severity, characterized by GCS score, would interact with the genetic predisposition to predict hippocampus size. Low GCS scores of TBI subjects, recorded upon admittance to the hospital, interact with the presence of the val66met polymorphism to significantly predict smaller

hippocampus volumes. The genetic variation influences can worsen already detrimental effects of traumatic brain injuries on the hippocampus, as shown through BDNF's the interaction with the injury severity.

With the knowledge GCS score and BDNF genetic predisposition, a TBI subject's outcome and recovery can be specifically projected and treatment plans can be put in place.

Limitations

This study was greatly limited by small sample size. Having only 40 subjects increases the effects of each subject. There were only five healthy control subjects with the val66met polymorphism. There was large variability in standard deviations, specifically in the TBI Positive group. Outliers may have caused this wide range and a larger sample size would have been more predictive of the entire trend.

Future Studies

Given the significant results predicting the hippocampal size, future studies may delve further into this trend. This study can be replicated with a larger sample size to more fully understand the implications of the BDNF polymorphism on hippocampal volume. Other genes can be examined to see if they more significantly related to volume and atrophy following traumatic brain injury, such as, ApoE4 and TNF. In addition, supplemental neuropsychological tests may be used to evaluate other cognitive functions of TBI patients with and without genetic polymorphisms. The tests could further examine the impact of left hippocampal atrophy on verbal, working and episodic memory deficits.

Additionally, future research could use other imaging techniques to assesses and measure hippocampal volumes. fMRI studies can look into the functioning levels of the hippocampus in TBI subjects compared to healthy control subjects to see if a significant difference is present.

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