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14. ABSTRACT

The major goals and aims of this study are to investigate whether differences in cognitive outcome are related to mechanism of injury as well as white matter integrity using diffusion tensor imaging (DTI). We are also collecting and analyzing data in order to determine whether MR variables of interest are associated with psychosocial/clinical outcome, and whether there are group differences by mechanism of injury. Specifically, in the context of this study, we use novel, sophisticated MRI methods (e.g., quantitative diffusion tensor [DT] tractography) in order to characterize white matter changes seen within and across TBI subtypes, identify those at highest risk for poor outcomes, and gain knowledge about potential interventions to aid in recovery of brain functioning and cognition. In addition, we seek to identify the unique psychosocial challenges posed by differing mechanisms of injury as well as investigate the contribution of genetic factors (Apolipoprotein-E ε-4 [APOE ε4] and brain-derived neurotrophic factor [BDNF]) to brain integrity, neuropsychological functioning, and neurobehavioral outcome.

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INTRODUCTION

The aims and goals of this project were to examine whether TBI history (mild to moderate blast and blunt force TBI) is associated with white matter changes using diffusion tensor imaging (DTI), and whether Veterans with history of head injury demonstrate differences in cognitive and psychosocial outcome. Primary aims used novel, sophisticated MRI methods (e.g., quantitative diffusion tensor [DT] tractography) in order to characterize white matter changes seen within and across TBI subtypes, identify those at highest risk for poor outcomes, and gain knowledge about potential interventions to aid in recovery of brain functioning and cognition. In addition, in our exploratory aim, we sought to identify the unique psychosocial challenges posed by differing mechanisms of injury as well as investigate the contribution of genetic factors (Apolipoprotein-E ε-4 [APOE ε4] and brain-derived neurotrophic factor [BDNF]) to brain integrity, neuropsychological functioning, and neurobehavioral outcome.

BODY

Growth and Expansion: At the end of this award, we have many reportables to provide the DoD. We credit the DoD and this particular grant that was awarded 7 years ago to our strong productivity as well as, more generally, the overall success of our lab which grew considerably over the period of the award. We recently welcomed an exceptionally talented postdoctoral fellow to our lab (Victoria Merritt; she began formally in Summer 2017), and we have added key personnel (Michael Walsh) who came on board as a research assistant. We are grateful to the DoD that has allowed us to conduct this important research since 2009, and we are hopeful that we will be in position to continue this work in our next phase of hopeful funding.

Productivity:
We continue to be productive in our work within our laboratory. In 2017, we presented 8 total TBI studies (4 at the 45th Annual Meeting of the International Neuropsychological Society (New Orleans, LA, 2017), 1 study at the 37th Meeting of the National Academy of Neuropsychology (Boston, MA, 2017), 1 study at the Society for Neuroscience (San Diego, CA, 2017), and 2 studies at the 2017 UC San Diego Biological Sciences Annual Student Research Showcase (La Jolla, CA).

We also recently presented another 6 studies at the 46th Annual INS meeting in February 2018. Over the past year, we have published 2 manuscripts, with an additional 2 recently accepted and in press. We currently have 2 manuscripts under review, and an additional 5 relevant manuscripts in preparation (more detail can be found in the *Reportable Outcomes* section).

Recruitment:
During this 7th year of this project, we have recruited and tested roughly 16 participants who represent either combat controls or patients who have sustained mild to moderate TBI; 2 participants partially completed the study (e.g., completing only cognitive testing or scanning) or were excluded due to failed drug screens on the day of testing (e.g., recent drug abuse). There are an additional 68 participants that were screened, but not included given that they did not meet full inclusion criteria. We have conducted approximately 706 phone screens of potential subjects throughout the course of the study. To date, we have enrolled a total of 158 subjects. Our recruitment rate is typically about 1-2 subjects per month. Our

attrition rate is close to 0; our study subjects are informed in advance about the duration of the study, so they almost always complete both the cognitive assessment and neuroimaging sessions.

Due to challenges recruiting appropriate normal control participants and TBI participants who are blast-exposed only that meet our inclusion criteria, we requested and were approved for a third one-year NCE (approved 09/30/2016). In order to fulfill our SOW aims and to increase sample sizes (particularly with respect to rounding out our normal control and TBI blast-only samples to assist in group comparisons of the neuroimaging, neuropsychological, and other data) we have taken the following steps regarding recruitment: (1) we have recreated both our normal control and TBI-centered recruitment flyers to be more specific and easily understandable (2) we have focused our recruitment efforts on the VA TV advertisement, which, for each research study, displays the recruitment information for two weeks at time (due to the high number of studies, they rotate the advertisements for fairness) at this VASDHS location, as well as at other San Diego VA outpatient clinics; (3) we have also better utilized word-of-mouth referral with our current TBI and control participants by asking them to share our study information with Veteran friends who may qualify, emphasizing that a history of TBI is not necessary for participation; (4) we continue to share/obtain referrals through other similar VA research studies, and regularly receive new referrals from the VA TBI and Cognitive Rehabilitation Clinic. Although many participants end up being excluded for reasons such as substance abuse or metal shrapnel precluding MRI, this clinic has consistently served as an excellent source of new TBI Veteran referrals this past year; and (5) we have established collaborative efforts with other ongoing VA research studies, to better receive new referrals of both TBI and control participants.

KEY RESEARCH ACCOMPLISHMENTS

Total Sample Recruited Under DoD award:

Total recruited N=158
TBI: n = 95; Controls: n = 57

TBI Severity Breakdown:
88% Mild
10% Moderate
3% Severe

Average # of TBIs 2.61 (1.37)
Average # of Blast exposures 11.21 (44.25)

Most Significant TBI Types:
19% Blast
81% Blunt or Blast/Blunt

Statement of Work: Specific Aims and Work Accomplished

Specific Aim 1: To determine whether hippocampal atrophy and microstructural white matter changes can be detected in mild to moderate TBI and to assess differences by mechanism of injury

(blast vs. blunt force).

***Hypothesis 1a*: Collapsed across group, TBI participants will demonstrate poorer fractional anisotropy (FA) in TBI predilection sites (i.e., anterior and posterior limbs of the internal capsule, genu and splenium of the corpus callosum, fornix) as well as lower hippocampal volumes than normal control (NC) participants.**

We have previously published results showing that TBI participants with reduced executive functions demonstrated significantly decreased fractional anisotropy (FA) of prefrontal white matter, corpus callosum, and cingulum bundle structures compared with both TBI participants without reduced executive functions and military control participants (Sorg et al., 2014). We later showed that history of TBI, after adjusting for age, education, depression, and PTSD symptoms, significantly predicted lower FA values in both the genu of the corpus callosum and in the left cingulum bundle; FA also negatively correlated with processing speed (Sorg et al., 2016). Interestingly, this more recent study showed that, while FA was negatively associated with processing speed and executive functions, it was not associated with memory or PTSD symptom ratings. Specifically, both processing speed and executive functions were significantly correlated with FA values from multiple tracts, including those that differed between TBI and MC groups. In contrast, there were no significant associations between DTI scores and memory performance. Additionally, partial correlations adjusting for age demonstrated no significant associations between FA and PTSD total symptoms (assessed via the PCL-M total score) or PTSD symptom subtypes (all $p > .05$).

Table 4 from Sorg et al. (2016):

Significant Partial Correlations between Neuropsychological Domain Scores and DTI

		Executive Functions	Processing Speed
FA	AIC Right	.27*	ns
	Cingulum Left	.38**	.38**
	Cingulum Right	.27*	.33**
	Genu	ns	.44**
	Body	.37**	.43**
	Splenium	.36**	.31*
	PIC Left	.51***	.50***
	PIC Right	.49***	.27*
RD	AIC Left	ns	-.27*
	Cingulum Left	ns	-.40**
	Cingulum Right	ns	-.37**
	Genu	ns	-.45**
	Body	-.30*	-.48***
	Splenium	ns	-.36**
	PIC Left	-.30*	-.48***
	PIC Right	-.27*	-.28*
AD	Body	ns	-.29*
	PIC Right	.32*	ns

Results of partial correlation adjusting for age

*p<.05, **p<.01, ***p<.001, ns = not significant

Although we did not initially propose to examine effort, we noted as the study progressed that a high number of individuals with TBI frequently fail effort, or performance validity, tests. We therefore set out to study this directly within our sample and published a report investigating the relationship between poor effort and white matter integrity in our sample. Effort was assessed using the Test of Memory Malingering (TOMM), a stand-alone performance validity test, and the CVLT-II Forced Choice Recognition test, an embedded measure of performance validity. Interestingly, contrary to expectations, we found that mTBI veterans who failed performance validity tests demonstrated more overall white matter abnormalities than the other groups (i.e., those who passed effort testing or controls with no history of TBI) (Clark et al., 2016). Regional white matter analyses revealed abnormalities in the anterior internal capsule and cingulum of both TBI subgroups relative to controls. Moreover, compared with the TBI-passed group, the TBI-failed group demonstrated significantly decreased white matter integrity in the corpus callosum. Below we have listed a table highlighting relevant demographic information by group, as well as a table showing some of the main findings from Clark et al. (2016).

Participant Characteristics (Clark et al. (2016))

	Controls	**TBI-Passed**	**TBI-Failed**	***p*-value**
N	23	43	13	
Age	32.9 (7.9)	32.9 (8.2)	31.5 (8.5)	p = .86
* Gender (men: women)	16:7	38:5	12:1	p = .11
* Ethnicity				
Caucasian	19	17	9	
African American	1	5	0	p < .001
Hispanic	0	16	1	
Asian	0	2	3	
Other	3	3	0	
Years of Education	15.1 (2.0)	14.1 (1.6)	13.5 (1.9)	p = .02
WRAT Reading Standard Score	101.3 (16.9)	99.4 (14.4)	98.9 (8.0)	p = .85
Number of mTBIs	-	2.4 (1.3)	3.2 (2.0)	p = .11
*% Reporting Blast exposure	-	69.8%	69.2%	p = .97
# Self reported Blast exposures	-	4.1 (12.92)	4.8 (7.0)	p = .85
*% Reporting any LOC	-	53.5%	76.9%	p = .12
"Worst" TBI LOC duration in minutes	-	6.08 (9.3)	7.25 (7.0)	p = .75
Months Since Last TBI	-	64.37 (43.8)	34.00 (20.3)	p = .02
NSI Total Score	-	32.7 (17.4)	48.8 (10.8)	p = .01
BDI-II Total Score	3.0 (4.3)	19.1 (11.1)	28.3 (13.3)	p < .001
PCL-M Total Score	19.9 (4.1)	43.6 (17.0)	63.8 (10.4)	p < .001

Statistical Analyses:
One-way ANOVAs were used to examine whether there were group differences on white matter burden (WMB). The independent variable utilized in each analysis represented the three groups: MCs, TBI-Passed and TBI-Failed; the dependent variables were WMB indices of FA, RD and AD.

ANOVA showed a significant main effect of group, ($F(2,76) = 5.310$, $p = .007$, $\eta_p^2 = .12$), for FA-WMB. Follow-up contrasts demonstrated that the TBI-Failed subgroup had significantly greater FA-WMB (more ROIs differed from that of controls) than both the MC (Cohen's $d = 1.08$, $p = .006$) and TBI-Passed (Cohen's $d = .83$, $p = .018$) groups. However, no significant differences were observed between MCs and the TBI- Passed groups ($p > .50$). With respect to RD-WMB, ANOVA revealed there was no main effect of group ($F(2,75) = 2.040$, $p = .137$), nor did follow-up contrasts reveal any significant differences between the groups (all p's > .05). Finally, with respect to AD-WMB, results revealed no main effect of group ($F(2, 28.546) = 2.348$, $p = .114$), nor follow-up contrasts revealed there were any significant differences between the groups (all p's > .05). See table below.

White Matter Burden (WMB) by Group

DTI Index of WMB	Military Controls		TBI-Passed		TBI-Failed		MCs vs. TBI- Passed	MCs vs. TBI-Failed	TBI-Passed vs. TBI- Failed
	Mean (SD)		Mean (SD)		Mean (SD)		*p*-value		
FA WMB	1.30	(1.77)	1.77	(2.12)	3.69	(2.92)	.69	**.01**	**.02**
RD WMB	1.56	(2.59)	2.28	(2.36)	2.46	(2.50)	.16	.24	.97
AD WMB	.45	(0.85)	.73	(1.31)	1.69	(2.02)	.65	.14	.33

FA = Fractional anisotropy; RD = Radial diffusivity; AD = Axial diffusivity

We also published results (Clark et al., 2016) showing that TBI participants with poor clinical outcome (cognitive fatigue complaints) show reduced white matter integrity in a striato-thalamocortical circuit (anterior internal capsule) known to mediate fatigue. Additionally, we recently have a manuscript in press (Clark et al., 2017), showing subjective experience of fatigue was significantly associated with both global and regional thalamic morphometric changes. Specifically, greater levels of self-reported fatigue with decreased right and left thalamic volumes (p's < 0.05). Fatigue was also significantly associated with decreased anterior and dorsomedial volumes of the right thalamic body ($p < 0.05$). We observed a similar trend for the left thalamic body ($p < 0.10$). These findings build upon those from existing functional neuroimaging studies in those with history of TBI, providing further evidence for the neural basis of cognitive fatigue in head injured adults.

We have continued to expand upon our imaging analyses to include additional ROIs that include long-coursing fibers (e.g., superior longitudinal fasciculus) that may be vulnerable to shear/tensile forces and other tracts critical for cognition (e.g., uncinate fasciculus may be important for executive functioning and/or memory). Analyses are currently underway.

***Hypothesis 1b*: Given suggestions in the literature that DTI may be more sensitive to TBI-related diffuse axonal injury, we expect that, across mechanism of injury, white matter integrity for all regions of interest will be more strongly associated with and predictive of TBI status than hippocampal volumes.**

As would be expected given the milder TBI severity of our sample, hippocampal volumes (right and left) do not discriminate between groups (all p-values > .05).

Hippocampal Volume between groups (mild-to-moderate TBI vs Military Controls):

Tests of Between-Subjects Effects

Dependent Variable: Right-Hippocampus

Source	df	Mean Square	F	Sig.
Corrected Model	3	3784764.816	26.889	.000
Intercept	1	10307046.770	73.226	.000
IntraCranialVol	1	10473048.560	74.405	.000
age	1	85216.074	.605	.438
TBI_vs_NC	1	4767.118	.034	.854
Error	187	140756.746		
Total	191			
Corrected Total	190			

Tests of Between-Subjects Effects

Dependent Variable: Left-Hippocampus

Source	df	Mean Square	F	Sig.
Corrected Model	3	5511709.894	38.195	.000
Intercept	1	6974381.472	48.331	.000
IntraCranialVol	1	14991842.300	103.890	.000
age	1	50604.753	.351	.554
TBI_vs_NC	1	262450.635	1.819	.179
Error	187	144304.557		
Total	191			
Corrected Total	190			

Instead, as hypothesized, we have found a number of differences between our TBI and control groups on white matter integrity as reflected by DTI (as described above). We have also included specific analyses below from Sorg et al. (2016).

After adjusting for important confounds (i.e., age, education, and depression), and PCL-M score, TBI was a significant predictor of lower FA values in both the genu of the corpus callosum (p = .03) and in the left cingulum bundle (p = .01). With regard to mean RD, after adjusting for age, education,

depression and PCL-M score, TBI history was also a significant predictor of higher RD in the left cingulum bundle (p = .01), right cingulum bundle (p = .04), and the genu (p = .01). PCL-M total score was not a significant predictor of DTI values in any region in models with or without the TBI grouping variable included (p's > .23).

***Hypothesis 1c*: Since individuals with blast injury frequently experience concomitant damage related to acceleration-deceleration and CNS compromise secondary to other internal injuries (e.g., lungs), it is expected that, when directly compared to the blunt TBI subgroup, the blast TBI subgroup will show lower FA values for each white matter tract of interest.**

We first explored whether those with blunt vs. blast TBI differed on right and left hippocampal volumes After adjusting for PTSD (as assessed by total score on the PCL-M), age, Intracranial Volume, and lifetime total number of TBI's, preliminary analyses revealed significant differences between TBI subtypes (pure blast [n=18] and blunt [n=51]) for both left and right hippocampal volume. Given the small sample size as well as the complex nature of blast injury and distinguishing pure blast vs. blast/blunt injury, we are preparing a manuscript to investigate this more thoroughly.

Tests of Between-Subjects Effects

Dependent Variable: Left-Hippocampus

Source	df	Mean Square	F	Sig.
Corrected Model	4	1391049.785	15.270	.000
Intercept	1	2018019.665	22.153	.000
IntraCranialVol	1	4905393.450	53.849	.000
age	1	84606.593	.929	.339
Total_number_of_TBIs	1	389.000	.004	.948
blast_vs_blunt	1	635082.982	6.972	.010
Error	68	91095.829		
Total	73			
Corrected Total	72			

Tests of Between-Subjects Effects

Dependent Variable: Right-Hippocampus

Source	df	Mean Square	F	Sig.
Corrected Model	4	1262662.774	13.052	.000
Intercept	1	4375000.989	45.225	.000
IntraCranialVol	1	2799403.656	28.938	.000
age	1	121977.102	1.261	.265

Total_number_of_TBIs	1	59333.675	.613	.436
blast_vs_blunt	1	1357522.901	14.033	.000
Error	68	96738.709		
Total	73			
Corrected Total	72			

Next we explored FreeSurfer derived volumetric indices from the following cortical areas of the temporal lobe: superior, middle, and inferior temporal; banks of the superior temporal sulcus; fusiform; transverse temporal; entorhinal; temporal pole; parahippocampal. There were no significant differences between TBI subtypes on any of these volumetric neuroimaging variables (p's < 0.05).

The table below describes the findings for regional volume. We found similar results for regional cortical thickness:

Source	Dependent Variable	F	Sig.	Partial Eta Squared
pure_TBI_blast	lh_bankssts_volume	.055	.815	.001
	lh_caudalanteriorcingulate_volume	1.276	.262	.016
	lh_caudalmiddlefrontal_volume	.135	.714	.002
	lh_cuneus_volume	2.440	.122	.031
	lh_entorhinal_volume	2.226	.140	.028
	lh_fusiform_volume	.950	.333	.012
	lh_inferiorparietal_volume	.249	.619	.003
	lh_inferiortemporal_volume	.165	.686	.002
	lh_isthmuscingulate_volume	1.296	.259	.017
	lh_lateraloccipital_volume	.140	.709	.002
	lh_lateralorbitofrontal_volume	.983	.325	.013
	lh_lingual_volume	.130	.719	.002
	lh_medialorbitofrontal_volume	.247	.621	.003
	lh_middletemporal_volume	.523	.472	.007
	lh_parahippocampal_volume	1.380	.244	.018
	lh_paracentral_volume	.203	.653	.003
	lh_parsopercularis_volume	1.109	.296	.014
	lh_parsorbitalis_volume	.287	.593	.004
	lh_parstriangularis_volume	2.804	.098	.035
	lh_pericalcarine_volume	3.358	.071	.042
	lh_postcentral_volume	.093	.762	.001
	lh_posteriorcingulate_volume	.240	.626	.003

	lh_precentral_volume	.127	.722	.002
	lh_precuneus_volume	.053	.819	.001
	lh_rostralanteriorcingulate_volume	.001	.975	.000
	lh_rostralmiddlefrontal_volume	1.927	.169	.024
	lh_superiorfrontal_volume	.050	.824	.001
	lh_superiorparietal_volume	.344	.559	.004
	lh_superiortemporal_volume	1.800	.184	.023
	lh_supramarginal_volume	.274	.602	.004
	lh_frontalpole_volume	.060	.807	.001
	lh_temporalpole_volume	.562	.456	.007
	lh_transversetemporal_volume	2.069	.154	.026
	lh_insula_volume	.257	.614	.003
Source	Dependent Variable	F	Sig.	Partial Eta Squared
pure_TBI_blast	rh_bankssts_volume	3.278	.074	.041
	rh_caudalanteriorcingulate_volume	.411	.523	.005
	rh_caudalmiddlefrontal_volume	.331	.567	.004
	rh_cuneus_volume	1.260	.265	.016
	rh_entorhinal_volume	2.505	.118	.032
	rh_fusiform_volume	1.018	.316	.013
	rh_inferiorparietal_volume	.309	.580	.004
	rh_inferiortemporal_volume	.822	.368	.011
	rh_isthmuscingulate_volume	.005	.945	.000
	rh_lateraloccipital_volume	.479	.491	.006
	rh_lateralorbitofrontal_volume	.268	.606	.003
	rh_lingual_volume	3.055	.084	.038
	rh_medialorbitofrontal_volume	.011	.916	.000
	rh_middletemporal_volume	.384	.537	.005
	rh_parahippocampal_volume	.175	.677	.002
	rh_paracentral_volume	.065	.800	.001
	rh_parsopercularis_volume	.315	.576	.004
	rh_parsorbitalis_volume	1.477	.228	.019
	rh_parstriangularis_volume	.389	.535	.005

rh_pericalcarine_volume	1.681	.199	.021
rh_postcentral_volume	.024	.876	.000
rh_posteriorcingulate_volume	.034	.853	.000
rh_precentral_volume	.261	.611	.003
rh_precuneus_volume	.315	.576	.004
rh_rostralanteriorcingulate_volume	2.700	.104	.034
rh_rostralmiddlefrontal_volume	.198	.658	.003
rh_superiorfrontal_volume	.219	.641	.003
rh_superiorparietal_volume	.137	.713	.002
rh_superiortemporal_volume	2.062	.155	.026
rh_supramarginal_volume	.024	.879	.000
rh_frontalpole_volume	.220	.640	.003
rh_temporalpole_volume	.060	.807	.001
rh_transversetemporal_volume	3.810	.055	.047
rh_insula_volume	.016	.899	.000

Importantly, pure blast related injuries are unfortunately rare in our sample and thus power is quite limited. However, when we compare the small sample of individuals with pure blast TBI (n=21) to those with pure blunt force trauma we see no significant differences in FA across any ROIs. In general, our sample was exposed to blast multiple times throughout deployment. While the vast majority of these injuries did not result in a TBI, subconcussive blast injuries may also be playing a part in white matter alterations and we continue to explore ways to further examine this.

<u>Specific Aim 2</u>: To investigate whether differences in cognitive outcome are related to mechanism of injury as well as to hippocampal volumes and white matter DTI variables.

***Hypothesis 2a*: We expect blast injury to be related to greater diffuse brain effects than blunt force injury. Thus, in comparison to the blunt force TBI subgroup, the blast TBI subgroup will show more pronounced cognitive deficits, particularly in executive functioning, attention/working memory, and processing speed.**

Preliminary analyses show no significant differences across the groups on any measure: Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference, Fluency, and Trails subtests; California Verbal Learning Test-2nd Edition; Wechsler Intelligence Scale for Adults 4th edition (WAIS-IV) Coding, Symbol Search, and Digit Span subtests; the Rey-Osterrieth Complex Figure Task; Reading subtest of the Wide Range Achievement Test- 4th edition (all *p*-values > .05). <u>However, a few comparisons were significant (or showed a trend towards significance), with blast showing worse performance than blunt</u>

force TBI. See below for values gleaned from independent samples t-tests:

- WCST Perseverative Responses (p = .025)
- D-KEFS Category Fluency Animals (p = .062)
- WMS 4 VR Recognition (p = .084)
- CVLT Long Delay Free Recall (p = .061)
- CVLT Long Delay Cued Recall (p = .016)

Group Statistics

	Subject Type	N	Mean	Std. Deviation	Std. Error Mean
WCST perseverative responses raw	blast	19	10.16	6.627	1.520
	blunt	52	7.08	4.329	.600
DKEFS category fluency (animals) raw	blast	19	38.53	7.820	1.794
	blunt	55	42.89	8.890	1.199
WMS VR-IV recognition raw	blast	13	6.00	1.080	.300
	blunt	46	6.59	.717	.106
CVLT-II Long Delay Free Recall	blast	19	9.45	4.298	.986
	blunt	57	11.21	3.200	.424
CVLT-II Long Delay Cued Recall	blast	19	9.79	3.980	.913
	blunt	57	11.77	2.639	.350

**We plan to embark upon more in-depth analyses with the neurocognitive variables such as examining number of impaired scores in the battery, number of declined scores in the battery (i.e., number of scores that are significantly lower than estimated level of pre-morbid functioning), and neurocognitive performance variability*

We also presented an abstract at INS in February, 2018 in Washington, D.C., and are preparing a manuscript investigating potential associations between blast exposure and brain changes in Veterans with mild TBI. Within the mTBI group, we found those with blast exposure had thinner cortices of the bilateral middle frontal gyrus (MFG) and right inferior frontal gyrus (IFG) relative to those without blast exposure (p's < 0.05). Additionally, collapsed across the sample, thinner MFG cortices were significantly associated with poorer executive functioning (p's < 0.05), even after adjusting for age and PTSD. These findings suggest that Veterans with mTBI and blast exposure may be especially vulnerable to brain and behavioral changes.

Hypothesis 2b: Collapsed across group, hippocampal volumes will be positively associated with and predictive of memory performance in TBI. Additionally, in line with our preliminary DTI studies, we expect that anterior FA measures will be positively related with executive functioning and processing speed, whereas posterior FA measures will be positively associated with language and memory functions.

We examined whether hippocampal volume and temporal lobe variables were associated with cognition in the TBI group. Significant findings are listed below:

- Rey-O Percentile was significantly associated with right ($r = -.353$, $p = .029$) and left ($r = -.369$, $p = .023$) hippocampal volumes.
- Wechsler Memory Scale-IV recognition raw was significantly associated with right hippocampal volume ($r = .332$, $p = .039$). Better performance was associated with greater volume.
- Next, we created a memory composite variable and explored hippocampal volume associations. However, the memory composites did not appear to be as sensitive as examining individual tests of memory, as there were no significant correlations.

We are currently writing up findings relevant to this Aim that we presented at the International Neuropsychological Society conference (Denver, 2015) in which we investigated white matter DTI measures of frontothalamic structural connectivity (FTSC). We found that, although groups did not differ in measures of FTSC, FTSC was associated with mTBI severity. Additionally, within the mTBI group, right FTSC correlated with an executive function composite, while left FTSC correlated with higher levels of self-reported disinhibition and executive dysfunction.

Although not explicitly stated in our goals, we have examined associations between hippocampal volume and subjective memory complaints. We have completed and will be presenting an abstract at INS in February, 2018 in Washington, D.C., examining such associations in mild TBI. Importantly, we found objective memory performance was not significantly associated with subjective memory complaints. However, smaller left hippocampal volume was significantly associated with greater subjective memory complaint severity ($p=0.001$). There was a similar trend for the right hippocampal volume ($p=0.057$).

An abstract representing these findings is below:

Hippocampal Volume Independently Predicts Subjective Memory Complaints in Mild Traumatic Brain Injury

Kelsey A. Holiday, Alexandra L. Clark, Scott Sorg, Michael Walsh, John Strom, Lisa Delano-Wood, & Dawn M. Schiehser

Objective: Veterans with mild traumatic brain injury (mTBI) frequently report memory deficits, which are not always supported by objective neuropsychological testing and are often related to psychiatric symptoms, such as PTSD. Given that neuroimaging allows for examination of possible neural correlates of subjective memory complaints, the objective of our study was to examine the relationship between hippocampal volumes, objective memory performance, and PTSD symptoms on subjective memory complaints in Veterans with history of mTBI.

Participants and Methods: 64 Veterans with mTBI and optimal effort completed tests of objective verbal and visual memory (WMS-IV Logical Memory and Visual Reproduction), subjective memory (NSI-14), the PTSD Checklist, and structural MRI. FMRIB's Software (FSL) was used to obtain hippocampal as well as intracranial volume (ICV) estimates. Two hierarchical regression analyses were

conducted to examine whether right or left hippocampal volume, visual or verbal memory performance, and/or PTSD symptoms predicted subjective memory complaints controlling for ICV and age.

Results: Regression analyses demonstrated that although objective memory performance was not associated with subjective memory complaints, left hippocampal volume was significantly and independently associated with subjective complaints (p=.001), with smaller volumes relating to greater complaint severity; results revealed a similar trend for the right hippocampal volume (p=.057). As expected, PTSD strongly predicted subjective memory complaints (p's<.001).

Conclusions: In mTBI Veterans, subjective memory complaints are best accounted for by PTSD symptoms and hippocampal volume, but not objective performance. Importantly, smaller hippocampal volumes are independently associated with greater subjective memory severity, suggesting a neural basis of memory complaints in mTBI not accounted for by PTSD or objective memory performance. Future research is needed to elucidate the behavioral manifestations of subjective memory complaints in mTBI.

Subjective Memory: NSI-14: Neurobehavioral Symptom Inventory (NSI) is a self-report scale for assessing post-concussive symptoms following mTBI. NSI items are rated on a Likert-type scale from 0 (none) to 4 (very severe). NSI item 14 (NSI-14) addressed forgetfulness and was used as the outcome measure for the present study.

Objective Visual Memory: VRss: WMS-IV Visual Reproduction (VR) VR I & II scaled score (mean).

Objective Verbal Memory: LMss: WMS-IV Logical Memory (LM) I & II scaled score (mean).

Demographic & Injury Characteristics	Mean (SD)
Age (years)	31.6 (5.9)
Education (years)	14.1 (1.6)
Gender (M:F)	51 : 8
Handedness (R:L)	51 : 8
# of TBIs	2.5 (1.3)
Time Since Injury (Months)	77.3 (48.7) Median = 72.0
Primary TBI Type (Blast:Blunt)	30 : 29
Combat Exposure	68 %
Loss of Consciousness	59 %
Alterations of Consciousness	41 %
PTSD symptom severity (PCL-M Total Score)	41.9 (17.8)
Logical Memory (LM) ss	10.3 (2.8)
Visual Reproduction (VR) ss	10.7 (2.7)
NSI-14	2.2 (1.3)

	Variable	*SE*	*β*	*p*
NSI-14 Subj. Memory				
Block 1	ICV	1.077	.037	.783
F=.041	Age	.030	-.008	.953
Block 2	ICV	.931	-.109	.349
F=8.802	Age	.024	-.050	.651
	LMss	.054	.014	.908
	VRss	.055	.108	.343
	PCL-M Total	.008	.484	**.001***
ΔR^2 =.403	Bilateral Hippocampus Volume	.001	-.406	**.001***

Our plan is to continue data processing and explore whether history of TBI interacts with hippocampal volume to predict memory test performance. However, when we test the interaction between TBI history and hippocampal volumes on cognition and control for PTSD we only find trending associations. Our plan is to follow up by exploring the direct influence of PTSD. Additional alternative analytic avenues we plan to pursue include determining whether those with history of TBI who meet clinical criteria for PTSD differ from those who not meet criteria for this psychiatric disorder.

Specific Aim 3: To determine whether MR variables of interest are associated with psychosocial/clinical outcome and whether there are group differences by mechanism of injury.

***Hypothesis 3a*: Given greater sensitivity to microstructural damage, FA of white matter in anterior regions will be more strongly associated with psychosocial clinical outcome than hippocampal volumes across TBI subgroups. Thus, in comparison to NC participants, lower FA of anterior regions will be associated with greater levels of psychological distress (i.e., depression, anxiety, and post-traumatic stress related symptomatology) and poorer functional outcomes.**

Throughout the period of this award, we have repeatedly shown that anterior white matter is indeed a predilection site for TBI, which is consistent with several other studies in the literature. More recently, we demonstrated that TBI significantly predicted FA values in both the genu of the corpus callosum and in the left cingulum bundle; FA also negatively correlated with processing speed (Sorg et al., 2016).

Given that white matter in the *cerebrum* did not show tight associations with psychosocial outcome as hypothesized (see table below), we investigated white matter integrity in the brainstem and then examined associations with commonly experienced postconcussive symptomatology (e.g., vestibular symptoms such as dizziness and imbalance). Historically, DTI studies of the brainstem have been considerably limited given challenges specific to being able to properly and accurately image this region (small, densely packed fibers with bony artifact). In a study published within a special journal series (Delano-Wood et al., 2015), we found that FA of the corticospinal tract was significantly negatively associated with LOC duration in participants with TBI history in our sample. In addition, lower FA of certain tracts – most especially the pontine tegmentum – was significantly associated with increased PCS symptoms (i.e., vestibular symptoms) and poorer physical functioning, even after adjusting for PTSD symptoms. Trends were also observed between lower pontine tegmentum FA, bodily pain, and greater fatigue. Lower FA of corticospinal tract and medial lemniscus was significantly associated with poorer emotional well-being after adjusting for PTSD symptoms.

Control Variables			NSI total score
PTSD total score	NSI total score	Correlation	1.000
		Significance (2-tailed)	.
		df	0
	FORNIX_FA	Correlation	.083
		Significance (2-tailed)	.528
		df	58
	AIC_L_FA	Correlation	-.154
		Significance (2-tailed)	.240
		df	58
	AIC_R_FA	Correlation	-.069
		Significance (2-tailed)	.600
		df	58
	PIC_L_FA	Correlation	-.111

		Significance (2-tailed)	.397
		df	58
	PIC_R_FA	Correlation	.019
		Significance (2-tailed)	.886
		df	58
	CING_L_FA	Correlation	-.214
		Significance (2-tailed)	.101
		df	58
	CING_R_FA	Correlation	-.056
		Significance (2-tailed)	.672
		df	58
	GENU_FA	Correlation	-.143
		Significance (2-tailed)	.275
		df	58
	BODY_FA	Correlation	-.184
		Significance (2-tailed)	.159
		df	58
	SPLENIUM_FA	Correlation	-.129
		Significance (2-tailed)	.327
		df	58

More recently, we published a brief report showing Clark et al. (2016a) that white matter disruptions of the left anterior internal capsule is significantly associated with greater levels of cognitive fatigue in Veterans with history of mild-to-moderate TBI.

In addition, to exploring direct relationships between MR variables and psychosocial/clinical outcome, we also sought to characterize how those with history of TBI may differ from MCs.

-Schiehser et al. (2015; manuscript) examined the relationship between postconcussive symptoms and quality of life (QOL) in Veterans with mild TBI. Results showed that perceived QOL was significantly worse in Veterans with mild-moderate TBI than in controls. In the TBI group, QOL was predominantly associated with affective symptoms, and moderate to strong correlations with fatigue and depression were evident across all QOL areas. Multivariate analyses revealed depression and fatigue to be the best predictors of Psychological, Social, and Environmental QOL, whereas sleep difficulty best predicted Physical QOL in mild-moderate TBI. Veterans with post–acute mild-moderate TBI evidence worse QOL than demographically matched Veteran controls. Affective symptoms, and specifically those of fatigue, depression, and sleep difficulty, appear to be the most relevant postconcussive symptoms predicting QOL in this population.

-Kim et al. (2016; abstract) found that when examining the relationship between subjective complaints of neurobehavioral symptoms and executive dysfunction/disinhibition, mood, and objective performance on an Go/No-Go inhibition task in mTBI veterans, self-reported executive dysfunction/disinhibition and depression were negatively associated with task performance. Further analyses demonstrated that higher levels of self-reported depression was the only significant predictor of task performance over and beyond the effects of subjective cognitive complaints or PTSD symptomatology. These results indicate depression as a potential treatment target for neurobehavioral symptoms related to disinhibition in mTBI veterans.

-Hanson et al. (2016; manuscript) characterized alcohol use among mTBI veterans and examined its relationship to mTBI and psychiatric symptoms. The mTBI group reported more alcohol-related psychosocial problems (e.g., fights, poor judgment, physical injuries, emotional problems) relative to MCs (p<.03). Within mTBI, more lifetime alcohol-related psychosocial problems were associated with combat exposure, longer post-traumatic amnesia from blunt injury, and higher depression, anxiety, and PTSD symptoms, as well as neurobehavioral symptoms (ps<.05). Additionally, greater lifetime withdrawal symptoms were associated with poorer attention and visual learning (ps<.03), while recent alcohol-related psychosocial problems were associated with poorer executive functioning (ps<.03). Our findings suggest that lifetime alcohol-related psychosocial or withdrawal symptoms may affect post-concussive symptomatology and cognitive functioning in some veterans with a history of mTBI.

Hypothesis 3b: **Since blast injury may be associated with greater psychological trauma secondary to exposure to improvised explosive devices—as well as the higher prevalence of other orthopedic injuries—it is posited that, after controlling for injury severity, individuals with blast TBI will show greater levels of psychological distress (i.e., depression, anxiety, and post traumatic related symptomatology) and poorer functional outcomes (i.e., greater deficits in work status and quality of life) than blunt force TBI.**

Although those with TBI consistently show significantly greater levels of depression, PTSD, anxiety and substance use when compared to military controls, to date we have found no differences by blast and blunt force injury. We will continue to recruit "pure blast" participants who have experienced mild to moderate head injury and will investigate subscores within the scales we examined (Beck Anxiety Inventory, Beck Depression Scale, PCL-M, Modified Cognitive Fatigue Scale; WHO-QOL).

Analyses comparing mTBI vs. controls on psychological distress and functional outcomes:

Group Statistics

	TBI vs. MC	N	Mean	Std. Deviation	Std. Error Mean
BDI total score	TBI	92	21.41	13.444	1.402
	Military Control	58	5.90	8.402	1.103
BAI total score	TBI	92	13.38	10.328	1.077
	Military Control	57	3.61	6.670	.883
PTSD total score	TBI	92	45.73	18.784	1.958
	Military Control	57	23.81	11.760	1.558

PSQI global score	TBI	66	11.42	4.644	.572
	Military Control	51	6.24	3.993	.559
Modified Fatigue Impact Scale total score	TBI	68	41.56	20.101	2.438
	Military Control	52	18.13	17.365	2.408
SF-36 physical functioning scale	TBI	69	75.7971	22.72960	2.73632
	Military Control	52	87.4038	19.41524	2.69241
SF-36 role physical scale	TBI	69	43.8406	43.38732	5.22322
	Military Control	52	86.0577	28.61743	3.96852
SF-36 bodily pain scale	TBI	69	49.3333	24.33024	2.92902
	Military Control	52	78.5962	23.20603	3.21810
SF-36 general health scale	TBI	69	54.6522	23.06119	2.77624
	Military Control	52	80.9808	19.14188	2.65450
SF-36 vitality scale	TBI	69	35.5217	21.59017	2.59915
	Military Control	52	61.3462	21.16961	2.93570
SF-36 social functioning scale	TBI	69	51.9855	26.91817	3.24057
	Military Control	52	87.9808	21.43183	2.97206
SF-36 role emotional scale	TBI	69	42.9950	43.19812	5.20044
	Military Control	52	82.0513	33.30842	4.61905
SF-36 mental health scale	TBI	69	52.2319	20.44084	2.46079
	Military Control	52	80.3846	18.11960	2.51274

As displayed in the table below, mTBI participants endorsed a significantly greater degree of psychological distress and worse functional outcomes compared to military control participants (all $p <$ .01).

	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
BDI total score	7.869	148	.000	15.516	1.972
	8.699	147.996	.000	15.516	1.784
BAI total score	6.360	147	.000	9.766	1.536
	7.012	146.716	.000	9.766	1.393
PTSD total score	7.898	147	.000	21.921	2.775
	8.760	146.973	.000	21.921	2.502
PSQI global score-- sum of 7 component scores	6.364	115	.000	5.189	.815
	6.489	113.646	.000	5.189	.800
Modified Fatigue Impact Scale total score	6.704	118	.000	23.424	3.494
	6.836	116.197	.000	23.424	3.426
SF-36 physical functioning scale	-2.957	119	.004	-11.60674	3.92478

	-3.024	117.082	.003	-11.60674	3.83882
SF-36 role physical scale	-6.086	119	.000	-42.21711	6.93631
	-6.436	117.127	.000	-42.21711	6.55982
SF-36 bodily pain scale	-6.680	119	.000	-29.26282	4.38071
	-6.725	112.562	.000	-29.26282	4.35147
SF-36 general health scale	-6.678	119	.000	-26.32860	3.94261
	-6.854	117.844	.000	-26.32860	3.84108
SF-36 vitality scale	-6.568	119	.000	-25.82441	3.93190
	-6.586	111.095	.000	-25.82441	3.92096
SF-36 social functioning scale	-7.930	119	.000	-35.99526	4.53892
	-8.186	118.612	.000	-35.99526	4.39709
SF-36 role emotional scale	-5.416	119	.000	-39.05637	7.21077
	-5.615	118.925	.000	-39.05637	6.95559
SF-36 mental health scale	-7.870	119	.000	-28.15273	3.57729
	-8.005	115.830	.000	-28.15273	3.51700

In contrast, there were largely no significant differences between mTBI blast vs. blunt force injury groups on psychological distress and functional outcomes. The only exception to this was PTSD symptoms, where the mTBI blast group endorsed greater PTSD symptoms than the mTBI blunt force group.

Group Statistics

	Blast Exposure	N	Mean	Std. Deviation	Std. Error Mean
BDI total score	no	39	21.18	14.283	2.287
	yes	63	19.71	12.973	1.634
BAI total score	no	39	11.38	9.284	1.487
	yes	63	13.17	10.920	1.376
PTSD total score	no	39	39.08	17.982	2.879
	yes	63	47.05	18.790	2.367
PSQI global score-- sum of 7 component scores	no	31	10.16	4.298	.772
	yes	45	11.69	4.747	.708
Modified Fatigue Impact Scale total score	no	32	38.22	21.166	3.742
	yes	46	40.93	19.508	2.876
SF-36 physical functioning scale	no	32	75.7813	23.72930	4.19479
	yes	47	77.0213	21.48456	3.13385
SF-36 role physical scale	no	32	48.4375	44.42169	7.85272
	yes	47	46.2766	44.22605	6.45103
SF-36 bodily pain scale	no	32	54.8438	23.82613	4.21190

	yes	47	51.8723	26.92632	3.92761
SF-36 general health scale	no	32	55.9688	22.18469	3.92174
	yes	47	56.4255	23.74387	3.46340
SF-36 vitality scale	no	32	36.2500	24.32972	4.30093
	yes	47	37.4681	21.53344	3.14098
SF-36 social functioning scale	no	32	56.2344	26.19344	4.63039
	yes	47	54.5213	29.30647	4.27479
SF-36 role emotional scale	no	32	51.0420	40.59143	7.17562
	yes	47	43.2619	46.05967	6.71849
SF-36 mental health scale	no	32	52.0000	21.36020	3.77599
	yes	47	55.5745	20.77179	3.02988

	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
BDI total score	.533	100	.595	1.465	2.748
	.521	74.774	.604	1.465	2.811
BAI total score	-.851	100	.397	-1.790	2.104
	-.884	90.346	.379	-1.790	2.026
PTSD total score	-2.116	100	.037	-7.971	3.767
	-2.138	83.383	.035	-7.971	3.728
PSQI global score	-1.432	74	.156	-1.528	1.067
	-1.459	68.586	.149	-1.528	1.047
Modified Fatigue Impact Scale total score	-.584	76	.561	-2.716	4.650
	-.576	63.248	.567	-2.716	4.719
SF-36 physical functioning scale	-.241	77	.810	-1.24003	5.13730
	-.237	62.203	.814	-1.24003	5.23615
SF-36 role physical scale	.213	77	.832	2.16090	10.15410
	.213	66.538	.832	2.16090	10.16273
SF-36 bodily pain scale	.504	77	.616	2.97141	5.89541
	.516	71.777	.607	2.97141	5.75901
SF-36 general health scale	-.086	77	.932	-.45678	5.30081
	-.087	69.657	.931	-.45678	5.23213
SF-36 vitality scale	-.234	77	.816	-1.21809	5.20270
	-.229	61.161	.820	-1.21809	5.32576
SF-36 social functioning scale	.266	77	.791	1.71310	6.43893
	.272	71.406	.787	1.71310	6.30193
SF-36 role emotional scale	.773	77	.442	7.78007	10.07049
	.791	71.925	.431	7.78007	9.82994

SF-36 mental health scale	-.742	77	.460	-3.57447	4.81537
	-.738	65.478	.463	-3.57447	4.84130

We are currently finalizing a manuscript that examines the associations between employment, satisfactory social support, and cognitive functioning among Veterans with mild to moderate TBI (Moore et al.). We found that employment and stronger levels of social support, which are important for positive health outcomes, are associated with better global cognition, particularly within the domains of attention and processing speed and language. Analyses show no differences by mechanism of injury, however. Future studies are needed in order to explore whether engagement in pro-social activities over time provides a buffering role against cognitive decline in Veterans in the aftermath of head injury.

Genetic Exploratory Aim: Since genetic and neurotrophic factors have been implicated as being possibly involved in both risk for and recovery from complications secondary to TBI, an exploratory aim is of the current study is to investigate two of these factors (apolipoprotein-ε4 [APOE-ε4] and brain-derived neurotrophic factor [BDNF]) as they relate to brain integrity, cognition functioning, and clinical/behavioral outcome. It has been suggested that APOE ε4 may be associated with decreased transport of lipids, increased accumulation of beta-amyloid, increased brain inflammation, impaired brain perfusion after injury, and poorer repair.[56-59] Indeed, a recent meta-analysis showed that the presence of the APOE ε4 allele is associated with increased risk of poor long-term outcome at 6 months after injury,[60] and it has also been shown to be related to duration of post-traumatic coma,[61] poorer neurorehabilitation outcome post TBI,[62] impaired cognitive performance in relation non-ε4 positive patients with TBI,[63] and slower recovery rate than those without the ε4 allele over a two-year period.[64] However, effects of the APOE ε4 allele are controversial as some studies have not shown any associations with neurological or cognitive outcome in TBI.[65-67] Much less is known about the effect of BDNF as a possible neuroprotective factor in the context of TBI. BDNF is a critical regulator of activity-dependent synaptic plasticity,[68,69] and it has been shown to be involved in neuronal survival and growth.[70] It has also been associated with improving cognitive and neurological deficits due to ischemia.[71] To our knowledge, however, no study has investigated BDNF in mild to moderate neurotrauma, and studies investigating relationships between BDNF and white matter, cognition, and clinical outcome are needed in the literature. Thus, we plan to investigate both APOE ε4 and BDNF in our sample of patients with TBI in order to better understand contributions of these genetic factors to white matter integrity and neurobehavioral outcome in blast and blunt-force TBI. We expect that, consistent with histopathological findings in the literature, participants with the APOE ε4 allele will demonstrate poorer white matter integrity and cognitive/clinical outcome even after controlling for age, time since injury, and severity of injury. Additionally, we expect that higher levels of BDNF will be associated with higher white matter integrity in predilection sites as well as better cognitive and clinical long-term outcomes (adjusting for age, time since injury, and injury severity). It is hoped that data obtained will be used as pilot data for future grant applications to explore associations between APOE, BDNF and longer-term outcome in our sample.

Genotyping has been complete on 89 TBI and 58 Military Control subjects. With respect to BDNF, we have completed two studies (published abstracts) that focus on (1) cognitive outcome and (2) brain

structure associations. The first which stems from a presentation we gave at INS in 2016 is currently being finalized and prepared for journal submission. The second was presented at INS in 2017 in New Orleans.

(1) Brain-Derived Neurotrophic Factor (BDNF) Genotype is Related to Executive Function in Veterans with History of Mild Traumatic Brain Injury

Nicole D. Evangelista, Alexandra L. Clark, Madeleine L. Werhane, Scott F. Sorg, Dawn M. Schiehser, Russell Kim, Mark W. Bondi, Katherine J. Bangen, & Lisa Delano-Wood

Objective: Brain-derived neurotrophic factor (BDNF) plays a role in neurogenesis and synaptic plasticity of hippocampal and forebrain areas; however, its expression is largely influenced by genotype. Compared with individuals without a BDNF Met allele, research shows Met-allele carriers have abnormal BDNF secretion. However, whether BDNF genotype is related to cognitive outcome, and how any association is modified by history of traumatic brain injury (TBI), is unclear. We therefore sought to clarify the relationship between BDNF genotype and cognition in Veterans with and without a history of mild TBI (mTBI). **Participants and Methods**: 117 Veterans (mTBI=72, Military Controls [MCs]=45]) underwent BDNF genotyping and were divided into (1) Met+ (*n* = 34 mTBI; *n* = 12 MCs), and (2) Met- (*n* = 41 mTBI; *n* = 33 MCs) carrier groups. Participants completed psychiatric symptom inventories and were administered neuropsychological tests that were reflected as the following composite scores in analyses: memory (CVLT: Total Learning, and Short and Long Delay Free Recall) and executive function (DKEFS: Verbal Fluency Switching Total Switching, Trails Number-Letter Sequencing; and WCST Perseverative Responses). **Results:** ANCOVA, controlling for psychiatric symptoms, revealed a significant Group x Genotype interaction for the executive function composite ($p = .01$). Examination of simple main effects revealed TBI+/Met- carriers performed significantly worse than TBI+/Met+ carriers, but no such association was found across genotype in the MC group. No significant interaction was observed for memory performance. **Discussion:** Results show that BDNF genotype is associated with poorer executive functioning but not memory performance in our sample of Veterans with mTBI. Though the underlying mechanism remains poorly understood, Met- carriers may be especially vulnerable to executive dysfunction after neurotrauma. Future studies are needed to further explore the epigenetic implications of BDNF on cognitive outcome in the context of head injury.

(2) Brain Derived Neurotropic Factor (BDNF) Val66Met Moderates the Association Between PTSD and Cortical Thickness in Veterans with History of Traumatic Brain Injury

Nicole D. Evangelista, Alexandra L. Clark, Katherine J. Bangen, Scott F. Sorg, Madeline L.Werhane, Dawn M. Schiehser, & Lisa Delano-Wood

Objective: Post-traumatic stress disorder (PTSD) has been linked to cortical thinning of frontal and temporal regions in Veterans with history of traumatic brain injury (TBI). However, it remains unclear how brain-derived neurotrophic factor (BDNF)—a protein that plays a role in neuronal growth, maturation and maintenance—may potentially influence the effects of PTSD on the brain. We therefore sought to clarify the associations between BDNF genotype, PTSD, and cortical thickness in Veterans with history of mild TBI (mTBI). **Participants and Methods:** 59 Veterans with history of mTBI underwent BDNF genotyping and were divided into (1) Met+ carrier (n=29) and (2) Met- carrier (n=30)

subgroups. Participants also completed psychiatric symptom questionnaires (PCL-M) and structural MR imaging. Cortical thickness values were derived from parcellated regions of interest (ROIs) using FreeSurfer. **Results:** Analysis of covariance controlling for age, revealed a significant PTSD x Genotype interaction for cortical thickness in the cuneus, precuneus, and the rostral anterior cingulate (*p*'s <0.05). Examination of simple main effects revealed that Met+ carriers with greater PTSD symptomology demonstrated significantly thinner cortices relative to Met- carriers (*p*'s < .05). **Conclusions:** Results show that BDNF genotype differentially affects the relationship between PTSD and cortical thickness in Veterans with history of mTBI. Specifically, mTBI Met+ carriers—who may have lower levels of this critical neurotrophin available in the central nervous system— appear to be especially vulnerable to the negative effects of PTSD on cortical thickness of several brain regions. These findings suggest that BDNF genotype plays an important role in modulating brain structure in the context of comorbid TBI and PTSD. Future studies should further evaluate the epigenetic effects of BDNF on recovery and treatment outcomes in Veterans with comorbid TBI and PTSD.

Work focused on APOE-e4 genotype

Historically, we have had some difficulty with low numbers of APOE-e4's in our TBI group (the genotype is overrepresented in our control group). Within the last year, we have made considerable strides toward our stated goals of investigating APOE-e4 in the context of TBI. We have had several studies focused on the influence of APOE-e4 on cognitive and clinical outcomes in Veterans with and without history of TBI, including one presentation at the National Academy of Neuropsychology Annual Conference in Boston, and 2 manuscripts that are currently under review. We also will be presenting an abstract at the INS Annual Meeting in Washington, D.C. in 2018.

DTI Variables of Interest: Per the Aims and Hypotheses of the award, we examined 10 white matter ROIs within the TBI group but found no significant differences across APOE-e4 allele groups. However we do find a trend for the left anterior internal capsule which shows reduced FA and higher MD in the APOE-e4+ group (p = .051)

Neurocognitive Variables: We have one study completed, examining the influence of the APOE-e4 allele on neuropsychological functioning in post-acute (>1 year since injury) mild TBI. This study will be presented at the INS Annual Meeting in February, 2018, and the manuscript is currently under review. Results suggest the APOE-e4 genotype contributes to poorer memory and processing speed in mTBI. Specifically, collapsed across the sample (mTBI=53 and military controls=46), there was a significant main effect of ε4 status for the memory and speed composites (ε4+ < ε4-; *p*=.005-.040), as well as the total number of impaired scores (ε4+ > ε4-; *p*=.017). Within individuals with a history of mTBI only, the ε4+ group performed more poorly than the ε4- group on the memory (*p*=.048) and speed (*p*=.018) composites, and displayed a significantly greater number of impaired scores (*p*=.017). These associations remained significant even after adjusting for PTSD symptoms. Within the military control group, there were no significant differences across any of the cognitive variables between ε4+ and ε4- (all *p*>.05).

We are currently conducting more in-depth analyses such as examining neurocognitive performance variability

Behavioral/Psychosocial Variables: We have had one study presented at the 2017 National Academy of Neuropsychology Annual Conference in Boston, MA, and the resulting manuscript is currently under

review. We examined the relationship between APOE genotype and neuropsychiatric symptoms in Veterans with history of mild-to-moderate TBI. There was a significant main effect of group across all symptom measures (TBI>MC; all *p*-values <.001) on the Beck Depression Inventory-II (BDI-II) total score, the Beck Anxiety Inventory (BAI) total score, and the PTSD Checklist-Military Version (PCL-M) total score. There was no main effect of ε4 genotype (*p*=.152-.222), but a significant interaction of group by ε4 genotype across all measures (*p*=.027-.047). Specifically, ε4+ TBI participants demonstrated significantly higher symptom scores across all measures than ε4- TBI participants (*p*=.007-.015). However, ε4 status had no effect on the severity of psychiatric symptom scores for the military control participants (*p*=.585-.708).

We are now examining the symptom clusters associated with each questionnaire in order to get a better sense of what might be contributing to the group differences. Additionally, we plan to examine symptom inventory summary indices (investigate subscales) which should better inform how groups differ. Importantly, preliminary findings suggest that APOE-e4 status predicts the NSI total score and somatic symptom cluster above and beyond the PCL-M total score and total number of TBIs.

REPORTABLE OUTCOMES

Relevant Manuscripts Published or In Press in 2017:

Published in 2017:

1. Clark, A. L., Bangen, K. J., Sorg, S. F., Schiehser, D. M., Evangelista, N. D., McKenna, B., Liu, T. T., & Delano-Wood, L. (2017). Dynamic relationship between perfusion and white matter integrity across time since injury in Veterans with history of TBI. *Neuroimage: Clinical*, *14*, 308-315.

2. Werhane, M. L., Evangelista, N. D., Clark, A. L., Sorg, S. F., Bangen, K. J., Tran, M., Schiehser, D. M., & Delano-Wood, L. (2017). Pathological vascular and inflammatory biomarkers of acute- and chronic- phase traumatic brain injury. *Concussion*, *2*(1).

Manuscripts In Press:

1. Clark, A.L., Sorg, S.F., Holiday, K.A., Bigler, E.D., Bangen, K.J., Bondi, M.W., Evangelista, N.D., Schiehser, D.M., & Delano-Wood, L. (in press) Fatigue is associated with global and regional thalamic morphometry in Veterans with history of mild traumatic brain injury. *Journal of Head Trauma Rehabilitation.*

Manuscripts Under Review:

1. Merritt, V. C., Clark, A. L., Sorg, S. F., Evangelista, N. D., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (Under Review). Apolipoprotein E (APOE) ε4 Genotype is Associated with Elevated Psychiatric Distress in Veterans with a History of Mild to Moderate Traumatic Brain Injury. *Journal of Neurotrauma*.

2. Merritt, V. C., Clark, A. L., Sorg, S. F., Evangelista, N. D., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (Under Review). Apolipoprotein E (APOE) ε4 Genotype is Associated with Reduced Neuropsychological Performance in Military Veterans with Post-Acute Mild Traumatic Brain Injury. *Neuropsychology*.

Manuscripts In Preparation:

1. Moore, R.C., Fazeli, P.L., Zlatar, Z.Z., Clark, A.L., Delano-Wood, L., Eyler, L.T., & Schiehser, D.M. (In Preparation). Preliminary evidence for an association between employment, satisfactory social support, and cognitive functioning among veterans with mild to moderate traumatic brain injury.

2. Evangelista, N.D., Clark, A.L., Werhane, M.L., Sorg, S.F., Schiehser, D.M., Kim, R.T., Bondi, M.W., Bangen, K.J., & Delano-Wood, L. (In Preparation). Brain-Derived Neurotrophic Factor (BDNF) Genotype is Related to Executive Function But Not Memory Performance in Veterans with History of Mild Traumatic Brain Injury.

3. Clark, A. L., Sorg, S. F., Merritt, V. C., Bangen, K. J., Evangelista, N. D., Holiday, K., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (In Preparation). Blast exposure is associated with anterior cortical thinning in veterans with mild traumatic brain injury.

3. Sorg, S.F., Clark, A.L., Schiehser, D.M., Bondi, M.W., & Delano-Wood, L. (In Preparation). Brain Derived Neurotrophic Factor (BDNF) Genotypes Moderate the Relationship between PTSD symptoms and Cortical Thickness in Veterans with Mild TBI.

4. Sorg, S.F., Clark, A.L., Schiehser, D.M., Bondi, M.W., Jak, A.J., Hanson, K.L., Woods, S.P., & Delano-Wood, L. (In Preparation). Intra-Individual Variability in Tests of Executive Functions in Veterans with Mild TBI: Associations with White Matter Microstructure.

5. Sorg, S.F., Luc, N., Clark, A.L., Kim, R.T., Bondi, M.W., Schiehser, D.M., & Delano-Wood, L. (In preparation). Frontothalamic Structural Connectivity in Veterans with Mild Traumatic Brain Injury: Associations with Executive Functions.

6. Merritt, V. C., Clark, A. L., Crocker, L. D., Sorg, S. F., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. Examination of Intra-Individual Variability Across a Comprehensive Neuropsychological Test Battery in Military Veterans with and without a History of Mild Traumatic Brain Injury: An Exploratory Study.

The following published abstracts were completed and presented in 2017 with joint funding from the DoD and VA:

1. Kim, R.T., Sorg, S.F., Holiday, K.A., Delano-Wood, L., Meloy, M., Clark, A.L., Tran, M., Locano, E., Jak, A.J., Eyler, L.T., Schiehser, D. (2017, February). Brain Function and Task Performance Predict Self-Reported Disinhibition and Executive Function in Veterans with Mild-Moderate Traumatic Brain

Injury. JINS, 23(1), 260-61. Poster presented at the 45th Annual Meeting of the International Neuropsychological Society, New Orleans, LA.

2. Sorg, S.F., Clark, A.L., Werhane, M.L., Kim, R.T., Schiehser, D., Bondi, M.W., Delano-Wood, L. (2017, February). Elevated Intra-individual Variability on Tests of Executive Functions in Veterans with Mild Traumatic Brain Injury. JINS, 23(1), 270. Poster presented at the 45th Annual Meeting of the International Neuropsychological Society, New Orleans, LA.

3. Clark, A.L., Bangen, K.J., Sorg, S.F., Werhane, M.L, Schiehser, D., Bondi, M.W., Delano-Wood, L. (2017, February). Repetitive Mild Traumatic Brain Injury Moderates the Association Between Age and Cerebral Blood Flow of Medial Temporal Lobe Structures. JINS, 23(10), 254. Poster presented at the 45th Annual Meeting of the International Neuropsychological Society, New Orleans, LA.

4. Evangelista, N.D., Clark, A.L., Bangen, K.J., Scott, S.F., Werhane, M.L., Schiehser, D.M., Delano-Wood, L. (2017, February). Brain Derived Neurotropic Factor (BDNF) Val66Met Moderates the Association Between PTSD and Cortical Thickness in Veterans with History of Traumatic Brain Injury. JINS, 23(1), 255. Poster presented at the 45th Annual Meeting of the International Neuropsychological Society, New Orleans, LA.

5. Haque, S., Clark, A.L., Evangelista, N.D., Werhane, M.L., Sorg, S.F., Schiehser, D.M., Delano Wood, L. (2017, June). Lower Nucleus Accumbens Volume is Associated with Reduced Reward-Based Decision Making in Veterans with History of Mild Traumatic Brain Injury. Poster presented at the 2017 UC San Diego Biological Sciences Annual Student Research Showcase.

6. Lapira, K.M., Werhane, M.L, Clark, A.L., Evangelista, N.D., Sorg, S.F., Schiehser, D.M., Bondi, M.W., Delano-Wood, L. (2017, June). Apolipoproptein E-e4 Genotype and Pulse Pressure Interact to Affect Cortical Thickness in Brain Regions Vulnerable to Alzheimer's Disease in Veterans with Mild Traumatic Brain Injury. Poster presented at the 2017 UC San Diego Biological Sciences Annual Student Research Showcase.

7. Clark, A. L., Schiehser, D. M., Sorg, S. F., Bangen, K. J., Werhane, M. W., Holiday, K., Delano-Wood, L. (2017, August). Global and Regional Thalamic Morphometry is Associated with Fatigue in Veterans with History of Mild Traumatic Brain Injury. Abstract presented at the American Psychological Association 125th Annual Convention in Washington, D.C.

8. Merritt, V.C., Clark, A.L., Sorg, S.F., Evangelista, N.D., Bondi, M.W., Schiehser, D.M., Delano-Wood, L. (2017, October), Apolipoprotein E (APOE) e4 Genotype is Associated with Increased Psychiatric Distress in Veterans with a History of Mild-to-Moderate Traumatic Brain Injury. Accepted for presentation at the 37th meeting of the National Academy of Neuropsychology in Boston, Massachusetts.

In addition, we are pleased to report the following abstracts were accepted to be presented in February, 2018 at the International Neuropsychological Society's conference in Washington, D.C.:

1. Clark, A. L., Sorg, S. F., Merritt, V. C., Bangen, K. J., Evangelista, N. D., Holiday, K., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (2018, February). Blast exposure is associated with anterior cortical thinning in veterans with mild traumatic brain injury. Abstract accepted for paper presentation at the 46th Annual Meeting of the International Neuropsychological Society in Washington, D.C.

2. Holiday, K. A., Clark, A. L., Sorg, S. F., Walsh, M. J., Strom, J., Delano-Wood, L., & Schiehser, D. M. (2018, February). Hippocampal volume independently predicts subjective memory complaints in mild traumatic brain injury. International Neuropsychological Society, Poster Presentation, Washington, DC

3. Merritt, V. C., Clark, A. L., Sorg, S. F., Evangelista, N. D., Werhane, M. L., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (2018, February). APOE-4 genotype modifies the relationship between TBI history and neurocognitive outcome in military veterans. Abstract accepted for poster presentation at the 46th Annual Meeting of the International Neuropsychological Society in Washington, D.C.

4. Sorg, S. F., Clark, A. L., Campbell, L. M., Werhane, M., Holiday, K. A., Merrit, V., Walsh, M. J., Jak, A. J., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (2018, February). The Relationship Between Subjective and Objective Memory Performance, PTSD Status and Injury Severity in Veterans with History of Mild Traumatic Brain Injury. International Neuropsychological Society, Poster Presentation, Washington, DC.

5. Strom, J., Sorg, S. F., Holiday, K. A., Clark, A. L., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (2018, February). Associations Between Time Since Injury and Cognitive Recovery Across Mechanism of Traumatic Brain Injury: Preliminary Evidence for Protracted Cognitive Recovery in Blast-Related Neurotrauma. International Neuropsychological Society, Poster Presentation, Washington, DC.

6. Walsh, M. J., Sorg, S. F., Clark, A. L., Holiday, K., Evangelista, N. D., Delano-Wood, L., & Schiehser, D. M. (2018, February). Regional gray matter volumetric differences predict fatigue symptoms in veterans with mild traumatic brain injury. Abstract accepted for poster presentation at the 46th Annual Meeting of the International Neuropsychological Society in Washington, D.C.

7. Werhane, M. L., Lapira, K., Clark, A. L., Evangelista, N. D., Sorg, S. F., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (2018, February). Apolipoprotein E-ε4 genotype and pulse pressure interact to affect cortical thickness in brain regions vulnerable to neurodegeneration in veterans with mild traumatic brain injury. Abstract accepted for poster presentation at the 46th Annual Meeting of the International Neuropsychological Society in Washington, D.C.

CONCLUSION

As the award period ends, we have made considerable progress toward our stated goals as outlined in our Introduction above. Given greater collaborations with other VA TBI investigators, our laboratory has grown considerably and productivity has increased significantly. We have been especially productive this final year in regards to analyzing our genetic data. We continue to grow our lab while also rounding

out our data collection so that we can then embark upon large-scale studies to test many of the hypotheses set forth in the original proposal.

APPENDICES

PDFs of Published Manuscripts from 2017:

1. Clark, A. L., Bangen, K. J., Sorg, S. F., Schiehser, D. M., Evangelista, N. D., McKenna, B., Liu, T. T., & Delano-Wood, L. (2017). Dynamic relationship between perfusion and white matter integrity across time since injury in Veterans with history of TBI. *Neuroimage: Clinical*, *14*, 308-315.

2. Werhane, M. L., Evangelista, N. D., Clark, A. L., Sorg, S. F., Bangen, K. J., Tran, M., Schiehser, D. M., & Delano-Wood, L. (2017). Pathological vascular and inflammatory biomarkers of acute- and chronic- phase traumatic brain injury. *Concussion*, *2*(1).

3. Clark, A.L., Sorg, S.F., Holiday, K.A., Bigler, E.D., Bangen, K.J., Bondi, M.W., Evangelista, N.D., Schiehser, D.M., & Delano-Wood, L. (in press) Fatigue is associated with global and regional thalamic morphometry in Veterans with history of mild traumatic brain injury. *Journal of Head Trauma Rehabilitation.*

4. Merritt, V. C., Clark, A. L., Sorg, S. F., Evangelista, N. D., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (Under Review). Apolipoprotein E (APOE) ε4 Genotype is Associated with Elevated Psychiatric Distress in Veterans with a History of Mild to Moderate Traumatic Brain Injury. *Journal of Neurotrauma*.

5. Merritt, V. C., Clark, A. L., Sorg, S. F., Evangelista, N. D., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L. (Under Review). Apolipoprotein E (APOE) ε4 Genotype is Associated with Reduced Neuropsychological Performance in Military Veterans with Post-Acute Mild Traumatic Brain Injury. *Neuropsychology.*

Dynamic across time since injury in veterans with history of TBI

Alexandra L. Clark, Katherine J. Bangen, Scott F. Sorg, Dawn M. Schiehser, Nicole D. Evangelista, Benjamin McKenna, Thomas T. Liu, Lisa Delano-Wood

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Dynamic Relationship Between Perfusion and White Matter Integrity Across Time Since Injury in Veterans with History of TBI

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Key Words: perfusion, arterial spin labeling, ASL, cerebral blood flow, CBF, white matter microstructure, diffusion tensor imaging, DTI, traumatic brain injury, TBI, time since injury, white matter

ABSTRACT

Objective: Cerebral blood flow (CBF) plays a critical role in the maintenance of neuronal integrity, and CBF alterations have been linked to deleterious white matter changes. Although both CBF and white matter microstructural alterations have been observed within the context of traumatic brain injury (TBI), the degree to which these pathological changes relate to one another and whether this association is altered by time since injury have not been examined. The current study therefore sought to clarify associations between resting CBF and white matter microstructure post-TBI. **Methods:** 37 Veterans with history of mild or moderate TBI (mmTBI) underwent neuroimaging and completed health and psychiatric symptom questionnaires. Resting CBF was measured with multiphase pseudocontinuous arterial spin labeling (MPPCASL), and white matter microstructural integrity was measured with diffusion tensor imaging (DTI). The cingulate cortex and cingulum bundle were selected as *a priori* regions of interest for the ASL and DTI data, respectively, given the known vulnerability of these regions to TBI. **Results:** Regression analyses controlling for age, sex, and posttraumatic stress disorder (PTSD) symptoms revealed a significant time since injury x resting CBF interaction for the left cingulum ($p < .005$). Decreased CBF was significantly associated with reduced cingulum fractional anisotropy (FA) in the chronic phase; however, no such association was observed for participants with less remote TBI. **Conclusions:** Our results showed that reduced CBF was associated with poorer white matter integrity in those who were further removed from their brain injury. Findings provide preliminary evidence of a possible dynamic association between CBF and WM that warrants additional consideration within the context of the negative long-term clinical outcomes frequently observed in those with history of TBI. Additional cross-disciplinary studies integrating multiple imaging modalities (e.g., DTI, ASL) and refined neuropsychiatric assessment are needed to better understand the nature, temporal course, and dynamic association

between brain changes and clinical outcomes post-injury.

INTRODUCTION

Traumatic brain injury (TBI) has come to be known as the predominant injury of U.S. Veterans returning from the recent wars in Iraq and Afghanistan (Hoge et al., 2008). Of the nearly two million military service members that have been deployed since the beginning of these wars, estimates suggest that an astounding 15-25% of these individuals have sustained at least one TBI during deployment (Fortier et al., 2014; Hoge et al., 2008; Terrio, Nelson, Betthauser, Harwood, & Brenner, 2011; Warden, 2006). The vast majority of these injuries can be classified as either mild or moderate (Defense and Veterans Brain Injury Center, 2016), and are often the direct result of either blunt-force (i.e., direct blow to the head) or blast-related (i.e., pressure wave from an explosive device) trauma. While most Veterans who experience mild neurotrauma do not require immediate or emergency medical care at the time of injury, a host of troubling cognitive (e.g., executive dysfunction, attention and memory deficits) (Combs et al., 2015; Vanderploeg, Curtiss, & Belanger, 2005), post-concussive (e.g., headaches, dizziness, fatigue) (King et al., 2012; Lippa, Pastoerk, Benge, & Thornton, 2010; Vanderploeg, Belanger, & Curtis, 2009), and psychiatric symptoms (e.g, anxiety, depression) (Brenner, Vanderploeg, & Terrior, 2009) frequently emerge post-injury. Collectively, these enduring neurobehavioral symptoms contribute to considerable health care costs (Stroupe et al., 2013; Tanielian & Jaycox, 2008), and they play a fundamental role in frequently reported decreased quality of life (Schiehser et al., 2015), and increased rates of disability and unemployment observed in Veterans with history of head injury (Lippa et al., 2015). Importantly, although most individuals with mild TBI appear to fully recover within about one year post-injury, a subset of individuals—oftentimes referred to as the "miserable minority"—continue to experience long-term cognitive, psychiatric, and behavioral difficulties that persist for many years after the initial

head injury event (Bigler, 2013; Ruff, Camenzuli, &Mueller, 1996; Vanderploeg, Curtiss, Luis & Salazar, 2007). Unfortunately, the exact neuropathological mechanisms underlying the persistent sequelae of mild neurotrauma remain poorly understood since traditional neuroimaging techniques are generally insensitive to subtle neuropathological changes associated with mTBI, as conventional computed tomography (CT) and magnetic resonance imaging (MRI) scans have largely yielded normal results in individuals with persistent neurocognitive or post-concussive symptoms (Bigler, 2013a; 2013b; Brenner, 2011; McAllister, Sparling, Flashman, & Saykin, 2001).

The advent of more sophisticated neuroimaging technology, coupled with experimental animal modeling of TBI, has provided insight into pathophysiological mechanisms thought to underlie the negative health outcomes of Veterans with history of TBI. Biomechanical and animal models of TBI have demonstrated that direct, or primary injury, to neurons, glia, and vessels occurs during neurotrauma (Bigler & Maxwell, 2012; Blennow, Hardy, & Zetterberg, 2012; Chatelin et al., 2011; Kenney et al., 2015; LaPlaca et al., 2007; LaPlaca & Prado, 2011; Povlishock & Katz, 2005). Moreover, secondary pathophysiological cascades (i.e., neuroinflammation, edema, ischemia, Wallerian degeneration) exacerbate local injury sites and contribute to diffuse damage post-injury (Bigler & Maxwell, 2012; DeKosky, Blennow, Ikonomovic, & Gandy, 2013; Farkas & Povlishock, 2007; Johnson et al., 2013; Magnuson, Leonessa, & Ling, 2012). While more severe and heterogeneous pathology is observed in those with moderate or severe TBIs, the mechanisms underlying milder forms of neurotrauma remain less well understood, as precise modeling of mild TBI in experimental studies is challenging, and autopsy cases are exceedingly rare. Nevertheless, advanced MRI techniques (e.g., diffusion tensor imaging [DTI], arterial spin labeling [ASL]) have been utilized for in-vivo quantification

of neurotrauma-related brain changes in Veterans (see Wilde et al., 2015 for review). However, while some studies find robust brain differences in Veterans with history of TBI relative to those with no history of head trauma (Mac Donald et al., 2011; Miller, Hayes, Lafleche, Salat, & Verfaellie, 2016; Petrie et al., 2014; Ponto et al., 2016), others fail to detect any alterations (Jorge et al., 2012; Levin et al., 2010).

The inconsistent nature of neuroimaging findings following TBI may be partially explained by the heterogeneous nature of injury, or alternatively, differences in sample characteristics, scanning parameters, and analytic techniques utilized. However, oftentimes unconsidered are (1) the dynamic relationship *between* brain variables of interest and (2) how *time* since injury may factor into brain changes. With respect to the former, studies of normal and pathological aging have consistently demonstrated that cerebral blood flow (CBF) plays a pivotal role in the maintenance of white matter (WM) tissue integrity (Burzynska et al., 2015; Chen, Rosas, & Salat, 2013; O'Sullivan et al., 2002; Salat, 2014; Steketee et al., 2016). Reduced CBF has been demonstrated to not only precede, but also directly contribute to negative WM micro- and macro-structural changes in older adults (Bernbaum et al., 2015; Brickman et al., 2009; Promjunyakul et al., 2015; Promjunyakul et al., 2016; ten Dam et al., 2007). Importantly, while both CBF and WM changes have been independently examined within TBI (Delano-Wood et al., 2015; Ponto et al., 2016; Vas et al., 2016), few studies have explored relationships *between* CBF and WM within this population. This is especially critical given CBF reductions could serve to exacerbate or contribute to any trauma-induced WM alterations well beyond the time of initial injury.

Unfortunately, the temporal course of the neuropathological consequences of TBI remains poorly understood (Greve & Zink, 2009; Povlishock & Katz, 2005). However, there is

some evidence to suggest that both CBF and WM changes may differ depending upon phase of injury (Eierud et al., 2014; Niogi & Mukherjee, 2010). For example, though findings are mixed, fractional anisotropy (FA)—a marker of WM microstructural integrity derived from diffusion tensor imaging (DTI)—has been observed to be both elevated and decreased in various studies examining those with history of TBI in the acute phase of injury relative to those without history of head trauma (Croall et al., 2014; Ling et al., 2012; Mayer et al., 2011). On the other hand, decreased FA is more commonly reported in individuals with history of TBI in the chronic phase of injury (Miller et al., 2016; Wada, Asano, & Shinoda, 2012). Similarly, while studies vary in reporting either elevated or decreased CBF in the acute phase of injury (Doshi et al., 2015; Meier et al., 2015), decreased CBF is most commonly observed in those with history of TBI who are further removed for their initial injury when compared to controls (Fridley, Robertson, & Gopinath, 2015; Ge et al., 2009). While there is no general consensus as to what constitutes acute versus chronic phases of injury, most Veterans are many months to years removed from their initial injury (i.e., in the chronic phase) during assessment; although, there is considerable inter-subject variability in the time between injury and assessment within and across previous Veteran TBI studies (Delano-Wood et al., 2015; Jorge et al., 2012; Mac Donald et al., 2011; Miller et al., 2016). It is especially critical to take into account *time since injury* when relating CBF and WM integrity given there is some evidence—at least in the aging literature—to suggest that CBF changes may persist for some time before negative WM alterations are subsequently observed (Brickman et al., 2009; Promjunyakul et al., 2015; ten Dam et al., 2007).

Therefore, there is a critical need to not only consider how CBF and WM relate to one another, but also how this relationship may depend on time since a TBI event. The current study sought to to examine the link between resting CBF of the cingulate cortex and WM integrity of

the cingulum bundle—two largely overlapping neuroanatomical regions that are known to be especially vulnerable to TBI effects (Bigler, 2007; Wu et al., 2010). Clarification of such relationships may assist in providing insight into factors influencing disparate brain findings in the TBI literature and elucidate findings that show WM degeneration may evolve over time during the chronic phase of injury (Bendlin et al., 2008; Yeh et al., 2016). We hypothesize that (1) decreased CBF of the cingulate cortex will be associated with reduced WM integrity of the cingulum bundle and (2), that this association will become more pronounced the further removed individuals are from their injuries. Importantly, findings may assist in clarifying mechanisms underlying the poor long-term outcomes and increased risk for stroke and dementia observed in those with history of TBI (Barnes et al., 2014; Burke et al., 2013; Y. H. Chen, Kang, & Lin, 2011; Lee et al., 2013).

METHODS

Study participants were 37 Operation Enduring Freedom, Operation Iraqi Freedom, and Operation New Dawn (OEF/OIF/OND) Veterans with history of mild-to-moderate TBI (mmTBI) recruited from outpatient clinics and posted recruitment flyers at the VA San Diego Hospital (VASDH) in La Jolla, California. The institutional review boards (IRBs) at the VA San Diego Healthcare System (VASDHS) and University of California, San Diego (UCSD) approved the study, and all study participants provided written and informed consent. Neuropsychological testing, TBI history interviews, and completion of questionnaires occurred at the Veterans Medical Research Foundation building located on the VASDHS campus. All MRI scanning took place at the UCSD Center for Functional MRI.

TBI Diagnostic Procedure

The Department of Defense (DoD)/VA TBI Task Force criteria (2009) was used for diagnosis of mild or moderate TBI. The criteria for mild TBI include loss of consciousness (LOC) < 30 minutes, or alteration of consciousness (AOC) or post traumatic amnesia (PTA) < 24 hours, while the criteria for moderate TBI were LOC >30 minutes but < 24 hours, or AOC > 24 hours or PTA >1 day but < 7 days. Per Clark et al. (2016) trained graduate level and post-baccalaureate research assistants completed TBI history interviews. Each study participant was assessed for both military (i.e., during enlistment in the US armed services) and non-military (i.e., prior to or after discharge from the military) related head injuries. All reported military-related injuries also include assessment of whether the mechanism of injury was blunt or blast-related. For any injury that met diagnostic criteria for mild or moderate TBI, the date of occurrence was recorded and time since the most recent TBI and date of evaluation was calculated for use in subsequent analyses

The following exclusionary criteria were applied to the study sample overall: (1) severe TBI (loss of consciousness [LOC] ≥ 24 hours, alteration of consciousness [AOC] > 24 hours, or posttraumatic amnesia [PTA] ≥ 7 days); (2) prior history of major medical illnesses (e.g., myocardial infarction) or neurological conditions (e.g., multiple sclerosis, stroke); (3) current active suicidal and/or homicidal ideation, intent, or plan requiring crisis intervention; (4) current or past history of DSM-IV diagnosis of bipolar disorder, schizophrenia, other psychotic disorder, or cognitive disorder due to a general medical condition other than TBI; (5) DSM-IV diagnosis of current substance/alcohol dependence or abuse; (6) a positive toxicology screen as measured by the Rapid Response 10-drug Test Panel; and (7) any contraindications that prevented MRI scanning. Participants were included in the study if they were OEF/OIF/OND Veterans between

the ages of 18-65, completed neuropsychological testing, and received both DTI and MPPCASL sequences.

Health status, combat exposure, & symptom rating scales

All study participants completed a background health questionnaire and height, weight, and blood pressure was collected at the time of their study visit. Exposure to wartime stressors and combat situations while on deployment was assessed using the Combat Exposure Scale (CES; Keane et al., 1989). Symptom rating scales that quantified current levels of posttraumatic stress (PTSD Checklist [PCL-M]; (Weathers, 1993), depression (Beck-Depression Inventory-II [BDI-II]; (Beck, 1996), and neurological symptoms (Neurobehavioral Symptom Inventory [NSI]; King et al., 2012) were also completed.

Neuroimaging Data Acquisition

All participants were scanned on a 3-Tesla General Electric MR750 whole-body MRI system with an eight-channel head coil. T1-weighted Anatomical Scan: A sagitally acquired high-resolution T1-weighted anatomical scan was collected using a 3D FSPGR sequence with the following parameters: FOV = 24 cm, 256 x 192 matrix, TR = 8.1 ms, TE = 3.192 ms, flip angle = 12°, TI = 550 ms, bandwidth = 31.25 kHz, and 172 1.2 mm slices.

DTI: DTI images were collected via dual spin echo EPI acquisition (Reese, Heid, Weisskoff, & Wedeen, 2003) with the following parameters: FOV = 24 cm, slice thickness = 3 mm, matrix size 128 X 128, in-plane resolution = 1.875 X 1.875 mm, TR = 8000 ms, TE = 88 ms, scan time: 12 minutes. Forty-three slices were acquired with 61 diffusion directions distributed on the surface of a sphere in conjunction with the electrostatic repulsion model (Jones, Horsfield, & Simmons, 1999) and a b value of 1500 s/mm^2. Collection also included one T2 weighted image with no diffusion (b = 0). Distortions due to a lack of magnetic field

homogeneity were reduced via field map corrections.

Resting CBF: Time-of-flight angiogram was collected with a three-dimensional spoiled gradient echo sequence (FOV = 22 x 16.5 cm, slice thickness = 1 mm, 0.57 x .74 x 1 mm^3 resolution, TE = 2.7 ms, TR = 20 ms, flip angle 15°) in order to define the location for PCASL labeling. The imaging volume was prescribed to visualize arteries above the vertebral crossing, but below the basilar artery. Axial images were used to select the slice most perpendicular to bilateral vertebral and carotid arteries and this location was then set as the labeling plane in an effort to achieve optimal tagging efficiency for the whole brain PCASL scan.

Whole-brain ASL data was acquired during a resting state using an MPPCASL sequence. Importantly, MPPCASL mitigates the adverse effects of off-resonance fields and gradient imperfections on the inversion efficiency in traditional PCASL techniques (Jung, Wong, & Liu, 2010). In MPPCASL, the blood magnetization is modulated with multiple RF phase offsets, and the resulting signal is then fit to a model function to generate a CBF estimate. Parameters included 20 5 mm thick axial slices (1 mm gap), FOV = 24 cm, matrix 64 x 64, PCASL labeling duration = 2000 ms, post-labeling delay = 1600 ms, , TR= 4200 ms, TE = minimum, volumes = 60, scan time = 5 minutes. To achieve CBF quantification in physiological units (mL/100 g-min), a 36-s cerebrospinal fluid (CSF) reference scan was obtained to estimate of the magnetization of CSF (TR= 4000ms, TE = 3.3 ms, NEX = 9 90°excitation pulse which is turned off for first 8 repetitions to create PDW image contrast; Chalela et al., 2000). A 32-s minimum contrast scan was also acquired to adjust for coil inhomogeneities (TR = 2000 ms, TE = 11 ms, NEX = 2) during the CBF quantification step. Finally, a field map was acquired using a spoiled gradient echo sequence to correct for field inhomogeneities (TR = 500 ms, TE1 = 6.5 ms, TE2 = 8.5 ms, flip angle 45°, scan time = 1:10 min).

Neuroimaging Data Processing

T1-weighted Anatomical Image processing: T1 anatomical images were reconstructed and parcellated into regions of interest using FreeSurfer software (Dale, Fischl, & Sereno, 1999). Manual edits were performed to ensure proper region of interest (ROI) segmentation and gray and white matter differentiation.

DTI processing: DTI preprocessing utilized the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL) (Smith et al., 2004). Two field maps were utilized to unwarp EPI acquisitions, and all images were motion corrected and visually inspected occurred for quality control purposes. The FSL program *dtifit* was used for voxel-by-voxel calculation of the diffusion eigenvalues and to provide fractional anisotropy (FA), a directional measure of diffusion ranging from 0 (isotropic diffusion) and 1 (perfectly anisotropic diffusion) that is reflective of fiber integrity.

Tractography: TrackVis (Wang, Benner, Sorensen, & Wedeen, 2007), using the fiber assignment by continuous tracking (FACT) algorithm, was used to generate the left and right cingulum bundle for each participant. First, a color-coded map, seen by loading the principle eigenvector image in FSL, was generated to display each voxel's main orientation of diffusion. This information, in conjunction with a non-diffusion weighted map, allowed the rater to place seed points for fiber tracking. An initial seed was placed inferior to the cingulum gyrus and superior to the corpus callosum in the coronal plane. Next, three additional seeds in the anterior portion, the middle, and the posterior portion were placed following the description of Concha, Gross, & Beaulieu (2005) to generate the entire cingulum bundle for each hemisphere. Finally, mean FA was extracted from the length of each generated tract for use in statistical analyses. See Figure 1 for depiction of left cingulum bundle ROI used in analyses.

Resting CBF: Each subject's raw ASL data, field map, and anatomical data were uploaded for processing to the Cerebral Blood Flow Biomedical Informatics Research Network (CBFBIRN; cbfbirn.ucsd.edu;(Shin, Ozyurt, & Liu, 2013) established at the UCSD Center for Functional Imaging. Field map and motion correction, skull-stripping, tissue segmentation, and conversion to absolute physiological units of CBF (mL/100g tissue/min) were completed through CBFBIRN. Quantified CBF maps for each participant were downloaded to a local server where they were blurred to 4 mm full-width at half maximum. Next, T1 images and partial volume segmentations were registered to ASL space and down-sampled to the resolution of the ASL images using the Analysis of Functional NeuroImages (AFNI) package (Cox, 1996). A threshold was applied that removed values outside of the expected physiological range of CBF (<10 or >150; (Bangen et al., 2014), then whole brain gray matter CBF and regional gray matter CBF values from the Desikan-Killiany (2009) atlas were extracted. Mean perfusion of the cingulate cortex was calculated as the average of the following gray matter ROIs for each hemisphere with each region's contribution to the average weighted by the volume of the region: rostral and caudal anterior cingulate, posterior cingulate and isthmus of the cingulate. See Figure 1 for a lateralized depiction of the left cingulate cortex utilized in this study.

Statistical Analyses

Multiple linear regressions were performed to determine (1) whether there was an association between CBF of the cingulate cortex and WM microstructural integrity of the cingulum bundle and (2) whether this association was modified by time since injury. A median split for time since injury was conducted to dichotomize TBI participants into two groups. Chi-squared analyses were utilized to compare the groups in terms of categorical variables and analysis of variance (ANOVA) was used for continuous variables. All statistical analyses were

conducted using the Statistical Package for the Social Sciences (SPSS) version 21 (SPSS IBM, New York, USA).

RESULTS

Participant demographics, TBI characteristics, and symptom rating scales for the sample are presented in Table 1. Participants were predominantly young (*Mean age* = 33.38 years) male (89%) Veterans who were blast-exposed (49%) and experienced moderate levels of combat exposure (*Mean total score of* 16.23 on Combat Exposure Scale) while on deployment. With respect to TBI injury characteristics, a greater proportion of Veterans experienced loss (65%) versus alteration of consciousness during their most significant TBI, these injuries were predominantly mild in severity (81%), and on average many months had passed since their most recent head injury (*M* = 69.05). Symptom rating scales revealed participants endorsed sub-threshold levels of posttraumatic stress symptoms (*Mean PCL-M total score* = 47.57) and depressive symptoms were moderate in severity (*Mean BDI-II total score* = 21.14).

Resting CBF and WM Associations

A set of multiple linear regressions were performed for each hemisphere in an effort to determine if there was an association between resting CBF of the cingulate cortex and white matter microstructural integrity of the cingulum bundle. In each model age, sex, PCL-M total score, and resting CBF of the cingulate cortex were entered as predictors. Results revealed that neither the left ($\beta = .08$, $p = .67$) or right ($\beta = .03$, $p = .88$) cingulate cortex CBF predicted left or right cingulum bundle FA, respectively.

Resting CBF, Time Since Injury, and WM Integrity

A second set of multiple linear regressions were performed for each hemisphere to determine whether time since injury moderated the association between resting CBF of the

cingulate cortex and cingulum bundle FA. In the first model, FA of the left cingulum bundle was entered as the dependent variable; age, sex, PCL-M total score, resting CBF of the left cingulate cortex, time since injury, and an interaction term (resting CBF of left cingulate cortex X time since injury) were entered as independent variables. As shown in Table 2, results revealed there was a significant resting CBF of the left cingulate cortex X time since injury interaction on FA of the left cingulum bundle ($\beta = 7.05$, $t = 3.99$, $p < .001$). A median split for time since injury was conducted for further inspection of this relationship (See Figure 2). Examination of simple main effects revealed that there was a significant positive correlation between resting CBF of left cingulate cortex and left cingulum bundle FA ($r = .48$, $p = .04$, $n = 19$) in Veterans furthest removed from their time since injury (≥ 62 months). However, for Veterans whose injuries were more recent (< 62 months), there was no significant association between resting CBF of the left cingulate cortex and left cingulum bundle FA ($r = -.19$, $p = .46$, $n = 18$). Results did not differ when total number of TBIs was included as a covariate in a secondary set of analyses and total number of TBIs ($\beta = .13$, $t = .67$, $p = .51$) was not a significant predictor of FA of the left cingulum bundle in the model. When this set of analyses was performed for the right hemisphere, there was no significant resting CBF of right cingulate cortex X time since injury interaction on FA of the right cingulum bundle ($\beta = -.994$, $t = .442$, $p = .66$).

Group Comparisons for Phase of Injury

In an effort to further understand the significant association between resting CBF of the left cingulate cortex and FA of the left cingulum bundle, participant demographics, TBI injury characteristics, and symptom rating scales were compared for participants who were closer versus further removed from their time since injury (See Table 3). Results revealed the groups were comparable on all comparisons except for time since injury. Although not statistically

significant, there were a greater proportion of individuals in those further removed from their TBI whose injuries were moderate (rather than mild) in severity. However, when a secondary set of analyses where TBI injury severity was included in the original regression model results remained the same and TBI injury severity was not a significant predictor of FA of the left cingulum bundle ($\beta = .04$, $t = .25$, $p = .80$). Moreover, sensitivity analyses revealed that when those with moderate TBIs ($n = 7$) were excluded from this analysis entirely, the significant interaction of resting CBF x time since injury on FA of the left cingulum bundle remained ($\beta = 6.84$, $t = 3.58$, $p = .002$).

DISCUSSION

The current study explored (1) the association between neuroimaging biomarkers of CBF and WM, and (2) the potential influence of time since injury on this relationship in Veterans with history of mmTBI. Results showed an interaction between time since injury and CBF of the left cingulate cortex on WM integrity of the left cingulum bundle. Specifically, in Veterans who were furthest removed from their time since injury, decreased CBF was significantly associated with reduced FA of the cingulum region. These findings provide preliminary evidence for a dynamic association between CBF and WM that may also play a pivotal role in increased risk for negative health outcomes (e.g. stroke, dementia) commonly observed in individuals with history of TBI. It is possible that this dynamic association observed between CBF and WM may partially explain the mixed findings in the neuroimaging literature, particularly since the vast majority of existing studies have focused on a single neuroimaging modality.

While our understanding of the pathophysiological consequences of TBI has improved, the time course of brain changes post-injury remains less well understood. Recent evidence suggests that TBI-related brain changes are not static, but may continue to evolve many months

to years following the initial insult. For example, Venkatesan and colleagues (2015) utilized resting state functional MRI (rs-fMRI) to explore the trajectory of connectivity patterns between the acute and chronic phase of injury in individuals with history of moderate-to-severe TBI. Results revealed that, relative to controls, the TBI group not only demonstrated altered connectivity patterns, but that these differences *intensified* from the acute to chronic phase of injury. In the present study, CBF and WM associations were only evident in those furthest removed from injury. It may be that this association is a manifestation of pathological processes that are characteristic of more chronic injury phases. Alternatively, as the aging literature has shown, CBF reductions may need to persist for some time before WM alterations arise (ten Dam et al., 2007; Brickman et al., 2009; Promjunyakul et al., 2015). Importantly, we cannot ascribe our results to exact causal or directional etiologies given the cross-sectional nature of this study and future studies are needed to further elucidate the time course of these dynamic relationships. Moreover, given this sample reflects mild TBI, there is also a critical need to clarify to what extent the observed findings may apply to samples comprising primarily moderate or severe injuries.

Our finding of an association between CBF and WM in those most remote from their injury aligns well with existing literature demonstrating a pronounced co-variation between WM integrity and vascular function in both healthy and pathological aging samples (Burzynska et al., 2015; Chen et al., 2013; O'Sullivan et al, 2002; Stekette et al., 2016). Within the context of TBI, CBF may play an important role in identifying those at risk for secondary WM changes following injury. For example, in an emergency room sample with mild TBI, decreased CBF at baseline assessment (within hours of injury) was tightly linked with reduced WM integrity at follow-up (on average 5 months post-injury; Metting, Cerliani, Rodiger, & van der Naalt, 2013).

The establishment of a relationship between CBF and WM within the context of head injury is critical, as maintenance of vascular health may be a critical point of intervention in the prevention of additional brain damage in those with history of TBI. Indeed, population-based studies have demonstrated that history of TBI is associated with increased risk for stroke (Burke et al., 2013; Chen et al., 2011), which reportedly persists for many years following the initial trauma (Chen et al., 2011).

Capturing brain changes in mild TBI is difficult, and it is possible that other neuroimaging metrics not directly examined here (e.g., cortical thickness) may also influence the CBF-WM associations observed in the current study. For example, a study by Duering and colleagues (2012) used longitudinal MRI methods to study how subcortical infarcts influence cortical morphology post-stroke. They found that damage to subcortical white matter initiated a secondary neurodegenerative process within cortical gray matter. Moreover, structural changes in the form of cerebral atrophy have also been linked to CBF reductions in other clinical populations (Appleman et al., 2008; Wirth et al., 2016). Unfortunately, work teasing apart primary and secondary injury processes within the context of TBI is still in its infancy, and prospective and longitudinal study designs with well-characterized samples are needed to tease apart how brain variables may interact with one another, especially over time, and ultimately influence behavioral outcomes.

Our secondary analyses revealed that the observed CBF and WM relationship in those furthest removed from their injury was not driven by fundamental differences in psychological, post-concussive, health, or injury characteristics relative to those whom were closer in time to their injury. Interestingly, both CBF and WM alterations have also been observed in those with elevated vascular risk in mid-to-late life (Beason-Held et al., 2012; Bangen et al., 2014; Maillard

et al., 2015; Wang et al., 2015); however, it is unclear to what extent TBI may increase the prevalence of vascular risk factors and whether individuals with elevated vascular risk are uniquely vulnerable to negative brain changes post-TBI. Future studies that include more comprehensive assessment of vascular risk are needed to understand how history of TBI and vascular risk factors may interact to affect the brain, cognition, and functional outcomes post-injury.

To our knowledge, this is the first study to investigate both ASL and DTI in the context of military TBI. However, there are several limitations that warrant discussion. Given the cross-sectional nature of this study, we cannot determine causal relationships between reduced CBF and reduced FA. Secondly, we were unable to explore whether these associations differ across mechanism of injury (i.e., blast versus blunt) or with blast-exposure given sample size restrictions. As is common with military studies of TBI, diagnosis of mild or moderate TBI was based entirely upon retrospective self-report of injuries and may therefore be subject to recall bias. We chose to examine CBF and WM of two closely linked neuroanatomical regions that are known to be vulnerable to the effects of neurotrauma; however, future studies will need to examine these effects across the brain and with other DTI metrics (i.e., axial and radial diffusivity) to further elucidate CBF and WM relationships. Replication with with larger sample sizes and longitudinal designs are also needed to provide more insight into the complex associations between WM integrity and CBF at different stages post-TBI.

CONCLUSION

Taken together, results indicate that, even after adjusting for psychiatric symptomatology, an association between CBF and WM exists in those with history of mmTBI. Although the exact nature and timeline of brain changes post-TBI is unclear, CBF and WM alterations may play a

pivotal role in the increased risk for negative health outcomes (e.g. stroke, dementia) that are observed in individuals with history of TBI. Currently, there is an ever-pressing need to consider how brain changes may differ with time and what might mediate or moderate these changes following injury. These findings contribute to our understanding of the possible dynamic relationship between CBF and white matter integrity, and they enhance our understanding of potential pathophysiological mechanisms that exist in the post-acute phase of injury.

Compliance with Ethical Standards & Disclosures. All procedures involved in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975. Informed consent was obtained from all patients included in the study. Alexandra Clark, Katherine Bangen, Lisa Delano-Wood, Scott Sorg, Dawn Schiehser, Nicole Evangelista, and Thomas Liu declare no conflicts of interest.

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Table 1. Participant Characteristics

	Mean (SD)
Age	33.38 (6.05)
Education	14.54 (1.56)
WRAT-4 Reading Standard Score	102.63 (12.45)
Gender (% Male)	89.2%
Ethnicity Caucasian African-American Hispanic Asian Native American	 35.1% 8.1% 37.8% 16.2% 2.7%
Combat Exposure Scale	16.23 (12.06)
PCL-M Total Score	47.57 (18.36)
BDI-II Total Score	21.14 (12.52)
NSI Total Score	37.11 (18.96)
Months Since Injury (Months)	69.05 (38.09), Median = 62.00, Range = 148
Total # of TBIs	2.84 (1.48)
TBI Severity (% Mild)	81%
% Most Significant TBI with LOC	65%
Blast-exposed (% Yes)	49%
Pulse Pressure (n = 36)	45.65 (8.28)
Height (inches)	67.93 (3.30)
Weight (lbs)	193.03 (45.58)
Current Smoker (% Yes)	16%
APOE-ε4 Genotype (n = 35; %Yes)	31%

WRAT-4 = Wide Range Achievement Test-4th Edition; PCL-M = Post traumatic Stress Disorder Checklist; BDI-II = Beck Depression inventory 2nd edition; NSI = Neurobehavioral Symptom Inventory; APOE-ε4 = Apolipoprotein-ε4 carrier

Table 2. Multiple Linear Regression Models for Left Cingulum Bundle FA

	R^2	*F*	**Standardized β**	**t**	***p***
	.445	4.01			.005
Age			-.304	-1.90	.07
Gender			-.359	-2.55	.02
PCL-M Total Score			-.007	-.052	.96
Time Since Injury			-7.02	-3.97	.000
CBF of Left Cingulate Cortex			-1.19	-3.37	.002
CBF X Time Since Injury			7.05	3.99	.000

Table 3. Group Comparisons for Phase of Injury

	Post-acute Group Mean (SD) n = 18	Chronic Group Mean (SD) n = 19	*F or* X^2	*p*
Age	33.11 (1.71)	33.63 (4.79)	.07	.80
Gender (% Male) ^	83.3%	94.7%	1.29	.26
Ethnicity^ Caucasian African-American Hispanic Asian Native American	 33.8% 5.6% 33.3% 22.2% 5.6%	 36.8% 10.5% 42.1% 10.5% 0%	2.74	.34
Combat Exposure Scale	13.96 (11.97)	18.38 (12.09)	1.25	.27
PCL-M Total Score	51.28 (16.92)	44.07 (19.41)	1.44	.24
BDI-II Total Score	24.44 (12.11)	18.01 (12.39)	2.55	.12
NSI Total Score	42.30 (18.47)	32.11 (18.51)	2.86	.10
Months Since Injury (Months)	39.28 (15.97)	97.26 (30.57)	51.39	< .001
Total # of TBIs	2.89 (1.56)	2.79 (1.43)	.04	.84
TBI Severity (% Mild)^	88.9%	73.7%	1.44	.23
% Most Significant TBI with LOC	55.6%	73.7%	1.34	.25
Blast-exposed (% Yes)	44.4%	52.6%	.25	.62
Pulse Pressure (*n* = 18, *n* = 18)	46.22 (7.34)	45.08 (9.31)	.17	.69
Height	67.75 (3.93)	68.11 (2.70)	.75	.75
Weight	191.78 (48.92)	194.28 (43.37)	.87	.87
Current Smoker (% Yes)^	17%	16%	.23	.24
APOE-ε4 Genotype (%Yes)	35%	28%	.63	.63

^Likelihood Ratio; PCL-M = Post traumatic Stress Disorder Checklist; BDI-II = Beck Depression inventory 2nd edition; NSI = Neurobehavioral Symptom Inventory; APOE-ε4 = Apolipoprotein-ε4 carrier

Figure 1. Lateralized depiction of the regions of interested utilized in the current study. The left cingulum bundle fiber track is superimposed on the left cingulate cortex (red).

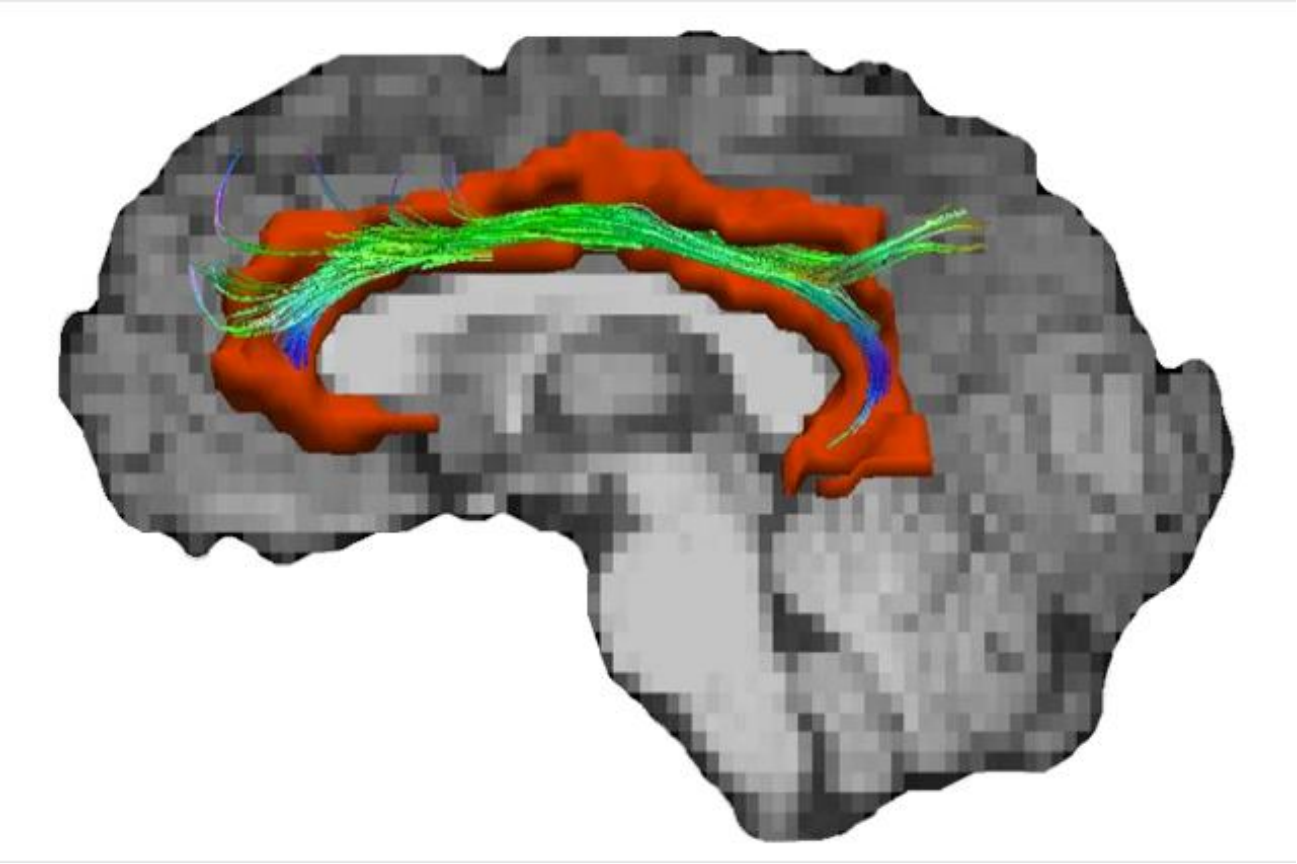
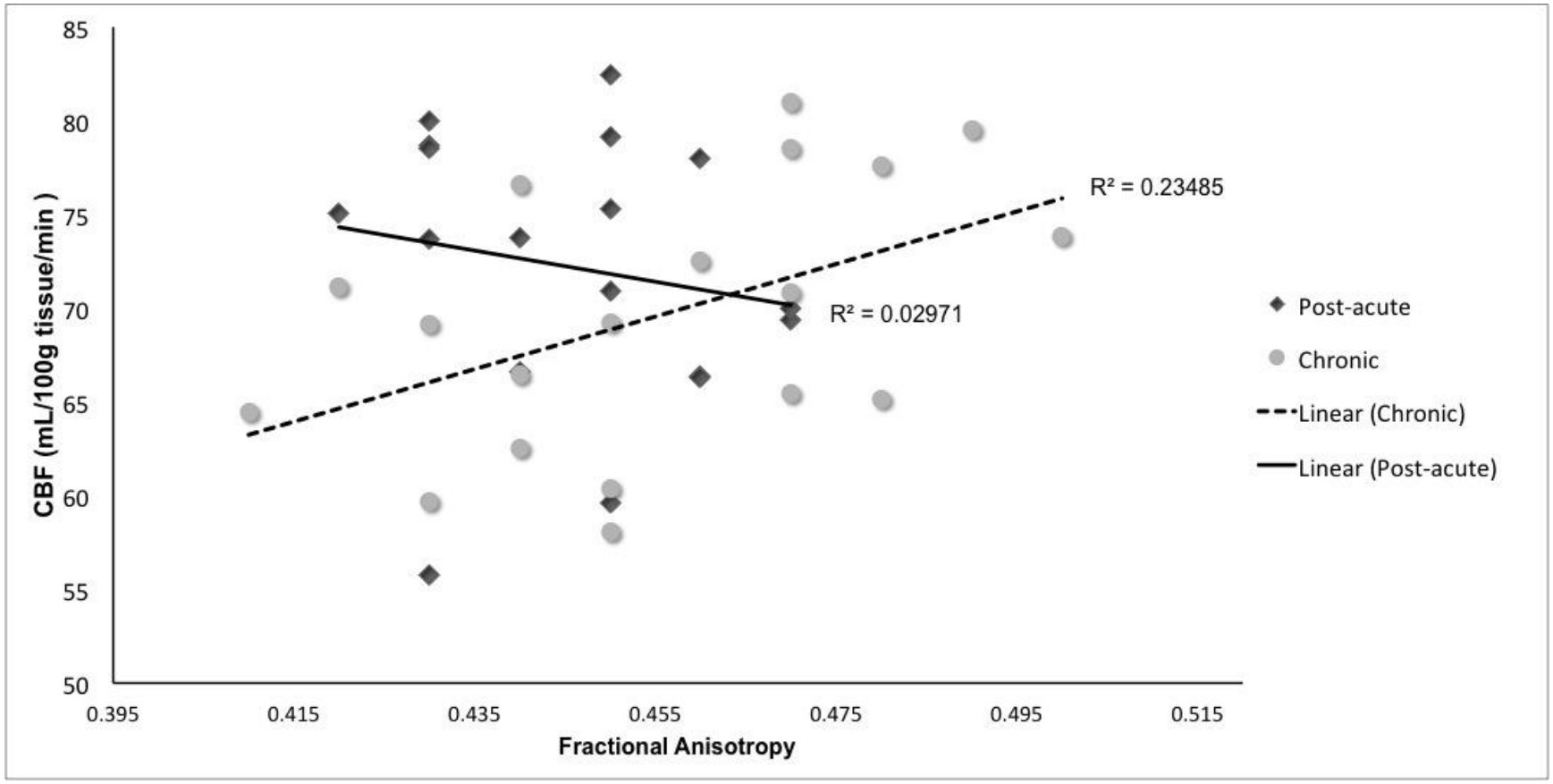


Figure 2. Cerebral Blood Flow (CBF) of Left Cingulate Cortex x Time Since Injury for Left Cingulum Bundle Fractional Anisotropy



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Highlights

- The inconsistent nature of neuroimaging findings following TBI may be partially explained by the heterogeneous nature of injury, or alternatively, differences in sample characteristics, scanning parameters, and analytic techniques utilized. However, oftentimes unconsidered are (1) the dynamic relationship *between* brain variables of interest and (2) how *time* since injury may factor into brain changes.

- Cerebral blood flow (CBF) plays a critical role in the maintenance of neuronal integrity, and CBF alterations have been linked to deleterious white matter changes. Although both CBF and white matter microstructural alterations have been observed within the context of traumatic brain injury (TBI), the degree to which these pathological changes relate to one another has not been examined.

- Reduced CBF has been demonstrated to not only precede, but also directly contribute to negative WM micro- and macro-structural changes in older adults (Bernbaum et al., 2015; Brickman et al., 2009; Promjunyakul et al., 2015; Promjunyakul et al., 2016; ten Dam et al., 2007). This is especially critical given CBF reductions could serve to exacerbate or contribute to any trauma-induced WM alterations well beyond the time of initial injury.

- The current study explored (1) the association between neuroimaging biomarkers of CBF and WM, and (2) the potential influence of time since injury on this relationship in Veterans with history of mmTBI. Results showed an interaction between time since injury and CBF of the left cingulate cortex on WM integrity of the left cingulum bundle. Specifically, in Veterans who were furthest removed from their time since injury, decreased CBF was significantly associated with reduced FA of the cingulum region.

- These findings contribute to our understanding of the dynamic relationship between CBF and white matter integrity, and may assist in clarifying mechanisms underlying the poor long-term outcomes and increased risk for stroke and dementia observed in those with history of TBI (Barnes et al., 2014; Burke et al., 2013; Y. H. Chen, Kang, & Lin, 2011; Lee et al., 2013).

Review

Concussion

Pathological vascular and inflammatory biomarkers of acute- and chronic-phase traumatic brain injury

Given the demand for developing objective methods for characterizing traumatic brain injury (TBI), research dedicated to evaluating putative biomarkers has burgeoned over the past decade. Since it is critical to elucidate the underlying pathological processes that underlie the higher diverse outcomes that follow neurotrauma, considerable efforts have been aimed at identifying biomarkers of both the acute- and chronic-phase TBI. Such information is not only critical for helping to elucidate the pathological changes that lead to poor long-term outcomes following TBI but it may also assist in the identification of possible prevention and interventions for individuals who sustain head trauma. In the current review, we discuss the potential role of vascular dysfunction and chronic inflammation in both acute- and chronic-phase TBI, and we also highlight existing studies that have investigated inflammation biomarkers associated with poorer injury outcome.

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Traumatic brain injury (TBI) is frequently associated with persistent behavioral, cognitive and psychosocial changes, many of which have important implications for daily functioning. Growing evidence furthermore suggests TBI should be conceptualized as a dynamic process that can affect brain health, directly and indirectly, several years after the initial insult [1–6]. Indeed, there have been repeated reports of persistent neurologic symptoms and an increased risk for the long-term development of neurodegenerative conditions, such as Alzheimer's disease (AD) and chronic traumatic encephalopathy (CTE) [7–12], in individuals who have sustained even mild forms of neurotrauma. Accordingly, there have been rapid developments in the clinical tools and measures used to identify and characterize neurotrauma both in terms of acute and long-term neuropathological effects of the injury. The use of biological markers, or biomarkers, for TBI diagnosis and prognosis may help clinicians more accurately identify when TBI has occurred, in addition to providing useful information about the underlying neuropathological mechanisms involved with poor injury outcome in the long-term. This review will therefore discuss evidence for the utility of fluid biomarkers in the identification of TBI. Specifically, in the context of both acute and chronic TBI, we will (1) underscore the role of vascular dysfunction and chronic inflammation in secondary injury following TBI, (2) highlight studies emphasizing inflammation biomarkers, and (3) discuss genetic factors associated with poorer injury outcome. When possible, the applicability of the available biomarker literature to mild forms of TBI is examined, with a particular emphasis on the need for future explorations of acute-

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and chronic-phase biomarkers across the entire TBI severity spectrum.

Characteristics of TBI & tissue injury mechanisms

TBI can be classified in many ways, including (1) the source of force causing the injury, (2) injury severity; and (3) mechanism of brain tissue damage. Penetrating TBI occurs when an impacting object penetrates all the protective layers surrounding the brain (i.e., skin, skull and meninges), directly inflicting injury to the brain tissue. This type of TBI is often complicated by hemorrhage, edema and inflammation, and is associated with a variety of poor outcomes (e.g., post-traumatic seizures, infection, cognitive and functional impairment, death). Given the severity and complexity of penetrating TBI, our current understanding of the pathophysiology and clinical outcome of penetrating TBI in humans has been largely limited to clinical case studies, observational studies utilizing small research samples and postmortem neuropathological studies. Recent advances in TBI animal research have demonstrated some promising rodent models of penetrating TBI that may further elucidate the pathophysiological mechanisms that underlie clinical outcome [13]. Comparatively, nonpenetrating or closed head trauma can be sustained through the application of both blunt and blast forces to the head. While the physics involved in the injuries sustained from these forces are distinct, the degree to which the resulting pathological processes and long-term clinical sequelae may differ remains unclear. In recent years, there has been an expansion in both human and animal studies attempting to model the neuropathology and clinical outcomes of nonpenetrating TBI.

American Congress of Rehabilitation Medicine guidelines suggest that TBI severity should be based on the presence and degree of an alteration of mental state (AMS), post-traumatic amnesia (PTA) and loss of consciousness (LOC). These criteria categorize head trauma using the following severity scale: absence of TBI is defined as a head injury that does not result in AMS, PTA or LOC; mild TBI (mTBI) is defined as a traumatically-induced physiological disruption of brain dysfunction, as indicated by any AMS, LOC of 30 min or less, PTA no greater than 24 h or Glasgow Coma Scale (GCS) of 13–15 h; and moderate TBI is defined as a head injury that results in an LOC of 30 min to 24 h, or AMS and/or PTA of greater than 24 h and fourth, severe TBI is a head injury that results in LOC greater than 24 h and/or PTA that lasts for more than 7 days [14]. Both primary and secondary mechanisms of head trauma have been identified. In contrast to primary injury, which describes the result of mechanical forces applied to the skull and brain at the time of impact, secondary injury, though poorly understood, is believed to represent damage to brain tissue that evolves over time [15,16]. Importantly, it is this mechanism of damage that is thought to underlie the long-term effects of mTBI.

Vascular dysfunction & TBI secondary injury

Injury mechanisms in TBI

There are important biophysical differences between blunt- and blast-force neurotrauma. Understanding these differences may serve a critical role in understanding neuropathological and clinical sequelae of TBI, especially along the mild end of the severity spectrum [17]. For example, blunt impact to the head may cause scalp tissue damage, fracture or depression of the skull, coup/countercoup impact of the brain tissue against the inner walls of the cranial vault and altered intracranial pressure (ICP) gradients [18]. Accordingly, fractures or depressions of the skull can displace underlying neural tissue (i.e., mass effect, creating localized displacement of neural tissue within the skull cavity). Similarly, coup/countercoup impact can result in focal contusions of cortical tissue, most commonly occurring in places in which the brain is most constrained or adjacent to ridged bony structures in the cranial cavity (e.g., anterior fossa, orbital sockets) [19]. Rapid acceleration/deceleration forces on the brain that are either linear or rotational in nature can also occur following blunt-force neurotrauma [20]. Theses forces stretch and deform brain tissue, exerting stress on neurons, glial cells and blood vessels, as well as altering membrane permeability. This ultimately results in damage to neuronal cell bodies, axons, dendrites, blood vessels and glial cells [21,22]. Focal and/or diffuse axonal injury (DAI) – characterized by enlarged axons with microtubule damage – is thus commonly observed following blunt-force mTBI. Interestingly, DAI tends to occur in brain regions with adjacent tissues of notably different densities (e.g., gray–white matter junctions). Such regions likely incur increased shearing stress due to the different rates at which the adjacent tissues move in response to the blunt-force impact.

The biomechanics of blast-force neurotrauma, while sharing some aspects in common with blunt-force trauma (e.g., force applied to head, which is loaded onto skull and brain tissue differentially), include some characteristics that render it both distinct and complex relative to other forms of neurotrauma. While many of these characteristics are related to the physics of the shockwave itself (e.g., blast overpressure and underpressure), an additional layer of complexity is involved with the environment in which blast-force

mTBI occurs (e.g., thermal heating, acoustic waves, radiation). While several disputed mechanisms have been put forth due to the additional complexity of characterizing the biophysics of blast-induced mTBI, many of these mechanisms center around the notion that exposure to a blast shockwave overpressure results in a distortion neural tissue and bodily fluids that have deleterious effects on the brain at both microbiological and gross morphological levels [23,24]. For example, it has been proposed that the quick changes in air pressure (shock wave) following an explosion lead to rapid acceleration and deceleration of neural tissues, exerting sheering forces that ultimately result in DAI in blast-induced mTBI [25,26]. Another proposed mechanism relates to disruption of blood–brain barrier (BBB), a highly selective vascular structure that controls the movement of molecules between peripherally circulating blood and CNS, due to the primary impact of the shockwave to the abdomen whereby kinetic energy from the shock wave is transferred into hydraulic pressure when it meets bodily fluids. This results in the rapid physical displacement of blood from the abdominal cavity to the cranial cavity, damaging small brain vessels and disrupting the BBB [15,27–30].

Common pathological sequelae in TBI

Irrespective of mechanism, both blast- and blunt-force neurotrauma generally appear to result in acute neural, glial and vascular damage with similar pathological sequelae. While damage to parenchymal tissue has historically garnered the most attention in TBI research, an increasing number of studies have highlighted the central role of cerebrovascular and alterations and dysfunction in both acute and chronic effects of neurotrauma (see [3–5] for reviews). Importantly, mounting evidence suggests that the acute vascular damage (e.g., torn or broken vasculature, microbleeds, endothelial cell damage, BBB damage, altered cerebral blood flood) and neuroinflammation (e.g., activation of microglia, gliosis and aggregates of activated macrophages) that occur from the immediate blunt or blast impact may trigger and perpetuate a host of secondary pathophysiological cascades (i.e., chronic neuroinflammation; edema; changes to the autoregulation of cerebral blood flood, neurovascular uncoupling and ischemia/hypoperfusion; hemosiderin deposits), ultimately promoting brain degeneration and dysfunction. Additionally, increased extravasation of peripheral immune cells, which are not normally found in the CNS due to their neurotoxicity in aggregate, may ultimately be promoted by decreased BBB in both acute- and chronic-phase neurotrauma [31]. Thus, while the complex molecular and cellular mechanisms responsible for the heterogeneous array of outcome following TBI are not fully understood, the presence of these chronic, insidious pathological processes may indeed be responsible for the poor long-term outcomes reported in some individuals following TBI.

Biomarkers of acute & chronic pathological processes following TBI

Markers of inflammation

It is well-documented that there are alterations of various neuroinflammatory process following neurotrauma [32]. In addition to increased immunoregulatory activity in CNS cells, peripheral immune cells and molecules have also been observed to cross the BBB in response to TBI [33,34]. Various pro- and anti-inflammatory agents, such as TNF, IL-1β, IL-6, IL-8 and IL-10, in particular, have been observed to fluctuate in response to TBI [35,36] and have therefore been investigated as putative biomarkers for TBI diagnosis and prognosis.

Tumor necrosis factor

Broadly, the tumor necrosis factor (TNF) superfamily refers to a group of cytokines involved in initiating and promoting cellular death. The TNF cytokine (previously referred to as TNF-α) represents a well-studied and highly versatile cytokine. While TNF is frequently studied in relation to its potent pro-inflammatory characteristics [37], it has also been observed to serve anti-inflammatory functions [38]. TNF is specifically expressed early in the response to neuronal injury and has a major role in initiating neutrophil and monocyte recruitment to the site of neuronal damage [39,40].

In previous studies employing animal models of TBI, increases in parenchymal levels of TNF have been detected as early as 1 h following TBI, and appear to peak 4–8 h following the initial injury [41–45]. The time course of TNF alteration in cerebrospinal fluid (CSF) differs from that in brain tissue, peaking at approximately 24 h following TBI [46]. Research employing animal models of mTBI specifically have suggested that TNF levels may not be sensitive to mild neurotrauma [e.g., 44]; however, here is some recent evidence to suggest that alterations in TNF levels can be detected in this subgroup. For example, one study found significant increases in TNF in rodents experiencing mild lateral fluid percussion injury as early as 3 h after the injury [47]. In another study, researchers observed significant increases in TNF in the hippocampus region of rodents induced with mild blast brain injury at 6 h post-injury [48]. A more recent study also reported that increases in serum levels of TNF were observed 4 h post-injury in a closed skull weight-drop model of mTBI [49].

In humans, elevated TNF concentrations in serum, plasma and CSF following TBI across the severity spectrum have also been reported [50–52], and appear to be increased in head-injured samples when compared with control groups [53,54]. Similar to animal models of TBI, CSF protein levels of TNF appear to peak within 24 h in the context of severe TBI [55], although some studies have reported multiple postinjury peaks of TNF levels when recorded over the course of several weeks postinjury [54]. In addition, TNF mRNA and protein, are among pro-inflammatory cytokines (i.e., IL-8, IL-1β) that have been observed to increase within minutes of TBI in postmortem brain tissue, indicating that a cerebral inflammatory cascade is initiated acutely following severe neurotrauma [56]. However, while this evidence suggests that TNF is involved in the neuroinflammatory response to severe TBI, research investigating TNF as a predictor of TBI outcome has produced mixed findings. Several small studies of severe TBI have reported no association between serum TNF levels and increased ICP, prognosis or mortality [55,57]. These studies are corroborated by a larger study reporting similar findings, with no relationship observed between initial TNF levels in both CSF and serum with GCS, ICP or neurological outcome in acute-phase severe TBI [58]. Conversely, more recent studies have reported an association between serum concentrations of TNF with increases in ICP, decreases in cerebral perfusion pressure and poorer 6-month outcome (extended Glasgow outcome scale [GOS]) in patients who sustained moderate or severe TBI [59,60]. A similar relationship was not observed for CSF TNF levels, suggesting that serum TNF levels may be more sensitive predictors of severe TBI outcome than CSF protein levels [60]. In sum, there is evidence that TNF is elevated in the acute phase on injury. This is largely derived from research employing animal or human models of moderate-to-severe TBI, although there are some data to suggest that acute elevations in TNF occur in mTBI as well. In addition, research aimed at characterizing the relationship between acute phase increases in TNF and outcome are mixed, and there appears to be no existing investigations on chronic-phase TNF elevations and associated outcome in humans.

Interleukin-1 beta

Interleukin 1 beta (IL-1β) is a highly regulated, potent pro-inflammatory cytokine that is released by macrophages and monocytes [61,62]. Although its primary role is the regulation and release of other cytokines, IL-1β is also involved in a variety of cellular activities, including cell proliferation, differentiation and apoptosis. It also has a reported role in certain brain pathologies that are common following head trauma (e.g., BBB damage [63], cerebral edema [64]), and has been implicated as having a role in certain chronic diseases that are prevalent in the aging population (e.g., cancer [65] and neurodegenerative disease [66,67]).

Previous research using both animal and human models of neurotrauma have reported an acute global increase in IL-1β mRNA, protein and activated caspase-1 (activated form of the IL-1β-converting enzyme) in postmortem brain tissue following TBI [56,68]. However, much more inconsistent findings have been reported regarding IL-1β levels in serum and CSF, with several studies reporting weak or no associations in severe TBI [69–72] and others reporting a significant increase following severe TBI [e.g., 73]. Despite this discrepancy in the literature, several studies have demonstrated the predictive value of serum and CSF levels of IL-1β as it relates to TBI outcome. High CSF and serum concentrations of IL-1β have been associated with poorer 3- and 6-month outcomes (i.e., GOS; recovery vs moderate–severe disability) as well as increased ICP following severe head trauma in both pediatric and adult populations [55,58,72,74–75]. Furthermore, in a more recent prospective cohort study, IL-1β was not only reported to be elevated over 3 months following TBI, but was significantly associated with increased odds of unfavorable outcomes at 6 months following severe head injury (GOS) [76]. Given such reported associations between acute and chronic IL-1β levels and outcome, some intervention trials using animal models of TBI have also explored IL-1β expression as a potential target for treatment of TBI. For example, Lee *et al.* [77] reported that pharmacologically induced hypothermia (PIH) was associated with decreases in mRNA expression of IL-1β and TNF were associated with improved sensorimotor functional recovery in mice after TBI. The goal behind such research has been to determine whether decreasing the levels or inhibiting the effects of pro-inflammatory processes positively affects TBI outcome. Findings from this line of research not only provide useful information regarding potential treatments for TBI but also support the notion that chronic inflammation may be the mechanism through which secondary neural injury following TBI may be sustained. But while this line of research appears promising for TBI interventions along the severe end of the injury spectrum, it remains unclear whether acute and chronic alterations in IL-1β expression occur following mTBI and, moreover, whether limiting the expression of this inflammatory marker can serve as a potential target for treatment of TBI.

Interleukin-6

Interleukin 6 (IL-6) is one of the most well-studied inflammatory markers across a variety of populations.

In the CNS, IL-6 is expressed by astrocytes, microglia and neurons [78–82]. In humans, IL-6 does not typically exist at detectable levels in serum under normal physiological conditions [83,84]; however, increases in IL-6 have been observed under pathophysiological conditions and are believed to be indicative of axonal damage [85,86]. There is also evidence for involvement of IL-6 in several normative and pathological physiological processes, including aging, TBI, inflammation, immunity and neural development [87,88]. Notably, IL-6 has also been associated with AD for which TBI and aging is considered to be a prominent risk factor [89,90]. Given these various associations between IL-6 and disease conditions, IL-6 is a particularly interesting target for studying the chronic effects of mild TBI.

IL-6 appears to be a highly sensitive biomarker for neurotrauma. While undetectable in the normal brain, rodent models of TBI reveal an acute increase in IL-6 expression following TBI [82,91]. In human, IL-6 concentrations have been reported to acutely, and sometimes persistently, increase following severe TBI [35,51,71,76,92]. This upregulation of the proinflammatory cytokine is easily detectable following acute TBI, although reports reflect some degree of variability in this response. CSF concentrations have been reported to increase significantly following TBI, reaching a maximum peak within 3–6-days postinjury [69,93]. Comparatively lower, but still detectable, alterations in IL-6 concentrations have been observed in both blood serum and plasma [35,74,94]. TBI severity has also been related to the intracranial IL-6 gradient in the blood of trauma patients at the time of hospital admission, with higher gradients associated with greater injury in severe TBI [95].

Given the pro-inflammatory role of IL-6 in the brain, the prognostic value of IL-6 levels following TBI has been investigated in several studies. In one study, elevated IL-6 serum levels within the first 17 h following severe brain injury effectively identified patients at risk of developing problematic levels of ICP [71]. Similarly, higher blood IL-6 intracranial gradients at the time of hospital admission were observed in brain trauma patients with fatal outcome in the 6 months following, compared with survivors [95]. More recently, Ferreira *et al.* [96] reported significant increases in IL-6 in nonsurvivors with severe TBI compared with survivors. In direct contrast, a study that used intracranial microdialysis to measure IL-6 concentrations in brain parenchyma reported that higher IL-6 levels were observed in survivors of severe TBI compared with nonsurvivors [94], a finding that suggests that IL-6 serves a neuroprotective function rather than as a risk factor for TBI poor outcome. These findings, however, conflict with other reports of IL-6 as a significant predictor of poor outcome following pediatric TBI (GCS and GOS) [97,98].

Although there is a clear relationship between increased neural expression of IL-6 expression head trauma, there are several characteristics of the cytokine that render it a poor predictive biomarker for TBI (when used in isolation). IL-6 is not exclusively expressed in the brain or in response to head trauma. Accordingly, IL-6 concentrations are sensitive to the presence of peripheral injuries, such as burns [99] and orthopedic injuries [71]. In addition, IL-6 had no prognostic value in predicting elevated ICP following severe TBI in patients with polytrauma, which was in stark contrast to its high sensitivity in individuals with TBI only [71]. An additional challenge with IL-6 is that its serum levels may be more indicative of BBB integrity than brain concentrations of the cytokine. This is suggested by the limited ability of IL-6 to cross the BBB [100], involvement of a transport mechanism to cross the BBB [101]. Thus, the presence of IL-6 following a possible TBI should be interpreted with caution. Taken together, both animal and human research in severe TBI suggest that IL-6 may be a sensitive (but not specific) biomarker for acute-phase TBI and associated outcomes, with more limited evidence for the role of IL-6 in the putative-protracted neuroinflammatory response thought to characterize chronic-phase TBI. Despite these findings, minimal human research has been conducted to explore the utility of the IL-6 biomarker in mTBI.

Interleukin-8

Interleukin-8 (IL-8), or CXCL8, is a member of a special class of small cytokines called chemokines. It is secreted by a variety of cells, including glial cells, macrophages and endothelial cells [102–104]. IL-8 is released from astrocytes in the presence of other cytokines that are acutely expressed following a TBI, such as TNF or IL-1β [105]. Once expressed, IL-8 induces chemotaxis and phagocytosis of neutrophils, attracting them to the site of neural damage and cleanup debris resulting from the injury [106]. While neutrophils typically leave the brain by 1 week following a brain injury, macrophages have been reported to linger for roughly 4 weeks [107]. This prolonged presence of activated leukocytes in the brain is neurotoxic and has been suggested to contributed to the ongoing neuronal damage that occurs following the acute brain injury. In addition to being studied as a potential biomarker for TBI, increased IL-8 expression has also previously been linked to cardiovascular disease [108], and it is known to be a potent promoter of angiogenesis [109]. This relationship between IL-8 and cardiovascular functioning

has important implications for both TBI and aging and furthermore may be reflective of a shared or synergistic relationship between the underlying pathological mechanisms involved with cerebrovascular disease, pathological aging and chronic TBI.

Along with several other pro-inflammatory cytokines, several studies have reported both acute and persistent increases in IL-8 levels following severe TBI [53,70,76,110–111]. The greatest increases in IL-8 concentrations are observed in CSF [110,111], but have also been observed to a lesser degree in serum after severe injuries [70,76,110,112]. While the increase in IL-8 is much greater in CSF compared with serum, several studies have demonstrated the prognostic value of blood-based IL-8 levels following head injury. For example, significantly lower acute plasma, and not CSF, levels of IL-8 have been observed in survivors of severe TBI, compared with nonsurvivors [96,113]. Similarly, serum IL-8 levels at 12 h [112] and up to 3 months [76] following TBI have been observed to be predictive of long-term functional outcome (i.e., GOS). These observational studies of TBI outcome have been further corroborated by human autopsy studies investigating the relationship between postmortem expression chemokines and antemortem TBI. For example, the upregulation of IL-8 mRNA and proteins was observed in postmortem in injured brains compared to controls [56]. Importantly, the overexpression of IL-8, as well as other chemokines, was associated with the presence of $CD68^+$ macrophages and GFAP-positive reactive astrocytes. In sum, the available studies on IL-8 alterations following severe TBI suggest that there exist both acute and chronic increases in the expression of this proinflammatory marker. Furthermore, it appears that increased IL-8 levels within both injury phases are associated with poorer injury outcome; however, like the available literature on other pro-inflammatory cytokines, there is a lack of studies aimed at characterizing the role of IL-8 in acute and chronic mTBI. Thus, while there is evidence to suggest that IL-8 may serve as a potential biomarker for acute- and chronic-phase severe TBI, additional animal and human research is needed to determine its utility as a biomarker for mTBI.

Interleukin-10

Contrary to the inflammatory markers previously covered in this review, interleukin 10 (IL-10) appears to act primarily as an anti-inflammatory cytokine. Importantly, IL-10 has an inhibitory effect on the production of several pro-inflammatory mediators, ultimately serving to regulate many of the cytokines that have been linked to acute and chronic inflammatory processes. Particularly relevant to inflammation following severe TBI is its effect of IL-10 on IL-1β and TNF, and interferon (IFN), all of which have been observed to exert detrimental effects on the brain [114,115]. Indeed, previous studies on the effects of IL-10 in normal physiological conditions, as well as in the treatment of certain pathological conditions, have implicated IL-10 as having a potential role in reducing the negative effects of neuroinflammation in TBI [116–121]. In addition, IL-10 expression appears to increase within the first 24 h following a severe head trauma [35,49,54,122], and, consistent with anti-inflammatory properties, this increase in IL-10 has been reported to correspond with a decrease in TNF levels. However, despite this well-documented anti-inflammatory role of IL-10, increased IL-10 following TBI has been repeatedly linked to poor outcome and mortality in both pediatric and adult severe TBI [58–59,96,123–125]. In addition, higher IL-10 levels measured at 10 or 30 h following severe TBI have also been found to be six- and five-times, respectively, more likely to result in hospital mortality compared with lower levels [125]. A possible explanation for this relationship is that the relative increases in pro-inflammatory cytokines compared with anti-inflammatory cytokines, rather than the individual increase in IL-10, is important in predicting TBI outcome. Recent findings from a prospective cohort study support this notion, where the ratio of pro-inflammatory burden relative to IL-10 was found to be associated with unfavorable outcome following severe TBI [76]. That is, higher levels of pro-inflammatory IL-6, relative to anti-inflammatory IL-10, were significantly associated poorer GOS scores at 6 months following severe TBI.

Research using animal models of head injury has also demonstrated the potential protective role of IL-10. For example, treatment of rats subjected to lateral fluid percussion-induced TBI with IL-10 has been shown to improve neurological recovery and reduced levels of IL-1β and TNF-α in brain tissues [126]. Similarly, local administration of IL-10 at the injury site attenuated the number and the hypertrophic state of reactive astrocytes and microglia and diminished TNF mRNA expression [127]. In a more recent study using a murine model of TBI, PIH following controlled cortical impact decreased mRNA expression of pro-inflammatory cytokines (TNF-α and IL-1β), but increased IL-6 and IL-10 levels [77]. Sensorimotor function was also improved in PIH, providing further evidence for the altering the ratio of pro- and anti-inflammatory cytokines, such as IL-10, as a potential target for improving TBI outcome. It should be noted that certain studies have reported, however, an association between IL-10 increases and mortality. Specifically, Ferriera *et al.* [96] reported significant increases in IL-10 levels in in nonsurvivors with severe TBI relative to survivors of the injury. Although there appears to be

converging evidence that IL-10 alterations occur during acute- and chronic-phase severe TBI, the predictive role of the biomarker for injury outcome remains unclear. In addition, there is limited research on IL-10 following mTBI within both the acute and chronic phases of injury.

Genetic factors

Although not always discussed in relation to biomarkers for TBI, the role of genetics in the identification of effective biomarkers for TBI diagnosis and prognosis is a critical consideration. That is, given that the environment in which acute and chronic pathological mechanisms following TBI occur is strongly influenced by genetic factors, it is necessary to understand how certain genes may influence the presentation or overall nature of these mechanisms. Although there are a multitude of genetic factors that affect brain structure and function, apolipoprotein-*E* (*APOE*) and brain-derived neurotrophic factor (*BDNF*) genes are the two most prominent in the TBI literature.

APOE

The APOE gene is one of the most widely studied genes in the context of neurotrauma and recovery [128]. ApoE is a protein largely associated with lipid and cholesterol transport as well as plasma lipoprotein metabolism in the CNS, all of which are essential for synaptogenesis [129]. Three *APOE* genetic polymorphisms encode one of the three isoforms: *APOE-ε2*, *APOE-ε3* and *APOE-ε4*. A loss in normal ApoE function has been observed in *APOE-ε4* carriers, negatively impacting synaptic plasticity and neuronal recovery from neurodegeneration [130,131]. The presence of the *ε4* allele has been characterized as a major risk factor for the development of AD [132,133]. This allele has also been linked to more abundant levels of amyloid-β plaque accumulation, which has been largely associated with AD [128]. *APOE-ε4* promotes neuronal cell death, resulting in accelerated neurodegeneration [134]. *APOE-ε4* has also been considered a major risk factor for various inflammatory metabolic diseases [134]. Relative to *APOE-ε2* or *APOE-ε3*, *APOE-ε4* has been associated with greater pro-inflammatory activity [129,135], increased numbers of APP-immunoreactive axonal varicosities and greater total human tau accumulation independent of injury status [136]. This indicates a potential primary effect of *APOE-ε4* on the severity of axonal injury in acute TBI.

As previously discussed, disruption of the BBB caused by TBI allows immune cells to cross into the brain, stimulating a cascade of inflammatory responses [15,27–30,137]. This subsequently results in a series of molecular events, including apoptosis, inflammation, microglial activation, altered plasticity and neuronal regeneration [21–22,138–139]. Microglial activation prompts perivascular macrophage production of cytokines integral in modulating secondary injury as well as recovery after injury [138]. Compared with *APOE-ε3* macrophages, *APOE-ε4* macrophages have demonstrated impaired efferocytosis, the process by which apoptotic or necrotic cells are removed through the process of phagocytosis, in mouse models [134]. *APOE-ε4* has also been found to potentiate endoplasmic reticulum stress and is associated with increased susceptibility to apoptosis in mice [134]. These findings indicate that *APOE-ε4* has a role in promoting macrophage dysfunction. This further suggests a possible mechanistic link between *APOE-ε4* and TBI secondary injury, as immune suppression following TBI has been found to slow brain infrastructure healing [140]. *APOE-ε4* expression is also associated with a reduction in cerebral vascularization, thinner vascular walls and decreased glucose uptake, compared with *APOE-ε2* and *APOE-ε3* expression [141]. This suggests an association between *APOE-ε4* expression and BBB disruption, providing a possible link between *APOE-ε4*-induced BBB anomalies and TBI secondary injury.

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is a polypeptide growth factor found in both the CNS and periphery. BDNF has been observed to serve a critical role in both neuronal survival and death within the CNS [142,143], as well as modulation of synaptogenesis and neurodevelopment throughout the human lifespan [144–147]. BDNF has been implicated in both normative neurocognitive functioning, as well as the pathophysiology in certain psychiatric conditions (e.g., bipolar disorder, post-traumatic stress disorder) [148–151]; however, recent research has suggested that polymorphisms in the *BDNF* gene may have a large influence on determining the role of BDNF in such conditions [152,153].

The most well-studied *BDNF* polymorphism involves the substitution of a single amino acid, Valine, with Methionine at codon 66 (*Val66Met*). The prevalence of this *BDNF* polymorphism, including both heterozygous ('*Val/Met*') and homozygous ('*Met/Met*') forms, has been estimated to be approximately 30–50% in the world population [154]. Importantly, the presence of the *Met* allele has been related to abnormal BDNF trafficking and activity-dependent secretion in neuronal cells [155,156]. Given the role of secreted BDNF in synaptic plasticity and neuronal survival in adulthood, the potential influence of *BDNF* genotype on mTBI outcome has been considered. With respect to TBI, the presence of the *Met* allele has been associated with better outcome following head injury, includ-

ing improved cognitive recovery [157–159], preserved general intelligence [160] and survival probability [161]. The presence of the *Met* allele has further been associated with better overall cognitive functioning following severe TBI [157,161]; however, evidence for whether this effect varies across cognitive domains is currently mixed [158,162]. The relationship between *BDNF* polymorphisms and TBI cognitive outcome is particularly interesting, given that it differs from observed effects in healthy individuals and other psychiatric conditions [157,160–161]. That is, the *BDNF* polymorphism appears to be protective under the pathophysiological conditions of TBI, but detrimental under other circumstances.

Taken together, the association between *BDNF* polymorphisms and cognition in the aftermath of neurotrauma appears to be complex. There is some evidence that the relationship between *BDNF* genotype and neuropsychological functioning in mTBI may differ from that of healthy individuals, where the presence of the *Met* allele may promote improved cognitive outcome under the pathological conditions following a head injury. Although *BDNF* genotype may not be a sufficient biomarker for long-term TBI outcome in isolation, future research on chronic TBI in individuals with different *BDNF* genotypes may reveal important differences in brain morphology and connectivity.

Conclusion

Given the necessity for an objective method of identifying and characterizing TBI, an extensive body of research evaluating putative biomarkers for TBI has developed over the past decade. Biomarkers of chronic mTBI, specifically, are important targets within this division of research given that they may help illuminate the underlying pathological processes that unfold over time after the initial stage of injury. Such information is not only critical for elucidating the poorly understood processes leading to poor long-term outcomes associated with milder forms of head injury, but it may furthermore help to identify possible targets for prevention or treatment of PCS and neurodegenerative diseases. Several potentially strong candidates have been identified in this review, most of which are associated with and/or reflective of both chronic vascular dysfunction (e.g., BBB breakdown) and neuroinflammation. This supports the notion that these mechanisms are likely involved with acute pathology following TBI and possibly the chronic pathology in response to secondary injury. Importantly, however, most of these biomarkers have only been investigated in moderate-to-severe TBI populations. Thus, while the available literature suggests that several pro- and anti-inflammatory cytokines may serve as useful biomarkers of acute- and chronic-phase moderate-to-severe TBI, the utility of these biomarkers for mTBI remains unclear.

There are several additional caveats to the use of inflammatory cytokines as biomarkers of acute- and chronic-phase TBI. First, while many of the described markers sensitive to acute- and chronic-phase moderate-to-severe TBI, most of them lack specificity. This is largely because inflammatory cytokines measured in blood serum or plasma are nonspecific to central inflammation (as peripheral injuries can also cause alterations in these markers). While CSF levels of these markers are excellent proxies indexing central inflammation, additional challenges exist with this respect in the TBI population due to the prevalence of BBB dysfunction. That is, BBB dysfunction may act as a confounder for CSF concentration of inflammatory proteins. However, while there exist clear limitations to the use of inflammatory cytokines as biomarkers for acute- and chronic-phase TBI, it should be noted that many of these challenges apply to most other potential fluid biomarkers. This highlights the need for careful consideration of the medium-specific sensitivity and specificity of all potential biomarkers for TBI, and emphasizes the need for future research aimed at clarifying the diagnostic and prognostic value of inflammatory biomarkers for different subclasses and phases of neurotrauma.

Future perspective

While the present review indicates the utility of certain biomarkers for acute and chronic phase in moderate-to-severe TBI, there are a lack of studies available related to these topics in mTBI samples, although research is increasing in this area. To date, several recent studies have demonstrated chronic vascular dysfunction in mTBI, supporting the notion that chronic-phase injury can exist even in mild forms of neurotrauma [15,27–30]. While notably less research has been conducted in the arena of chronic inflammation in remote mTBI, future work exploring the presence of chronic inflammation in mTBI should be prioritized given the empirical support for the presence of these processed in moderate-to-severe TBI. Indeed, such research may provide findings that not only critically aid in the identification of individuals at high risk for developing poor long-term outcomes following mild neurotrauma but may also inform the development of targeted interventions in order to reduce persisting symptomatology. Additionally, given that many of the reviewed biomarkers have limited utility when used in isolation, a multibiomarker approach should be considered and implemented in future research, as the combined characteristics of the above-mentioned biomarkers have the potential to

address many of these failures of individual biomarkers in the prediction of outcome in head-injured populations. Moreover, integrating multimodal biomarker approaches (e.g., fluid biochemical assays, neuroimaging, electrophysiological measures) may be particularly promising, given that different biomarker methods may provide varied methodological advantages and disadvantages with respect to acute- and chronic-phase TBI sensitivity and specificity. Lastly, since most of the discussed biomarkers have only been studied in the context of moderate and severe TBI during the acute phase of injury, future research is needed to test the utility of these biomarkers in the setting of the chronic effects of mild head trauma.

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Executive summary

Background

- Growing evidence suggests that traumatic brain injury (TBI) – even milder forms – should be conceptualized as a dynamic process that can affect brain health, directly and indirectly, several years after the initial insult. Thus, TBI research has begun to identify effective biomarkers for both the acute (i.e., primary injury sustained from the initial traumatic force) and chronic (secondary pathological processes that unfold after the initial brain injury that causes long-term changes to brain structure and function) phases of injury.

Characteristics of TBI & tissue injury mechanisms

- There are several different ways in which TBI can be classified, including (1) the source of force causing the injury, (2) injury severity, and (3) mechanism of brain tissue damage. Despite these differences, research to date suggests that certain primary and secondary pathological mechanisms are common across differing mechanisms of non-penetrating TBI.

Vascular dysfunction & TBI secondary injury

- The presence of chronic vascular dysfunction and neuroinflammation may characterize the chronic-phase TBI, and may be responsible for poor long-term outcomes reported in some individuals with head trauma histories.

Biomarkers of acute & chronic pathological processes following TBI

- Various inflammatory cytokines (i.e., TNF, IL-1B, IL-6, IL-8, IL-10) have been studied in acute- and chronic-phase TBI. These studies have evaluated the diagnostic and prognostic sensitivity and specificity of these markers. While not frequently discussed in conjunction with these biomarkers, genetic factors should be considered when evaluating the utility of acute and chronic biomarkers of TBI.

Conclusion & future perspective

- Several inflammatory cytokines appear to be sensitive diagnostic biomarkers for acute- and chronic-phase TBI, although most of the extant literature has focused on moderate-to-severe samples. Many of these markers, however, lack specificity to TBI, and furthermore have mixed prognostic value for injury outcome. Given the lack of research on this topic in samples with milder forms of head trauma, future studies should explore the presence of these pathological processes in acute- and chronic-phase mTBI. Finally, additional efforts to validate multibiomarker approaches are needed in order to assist in improving diagnostic specificity of TBI and enhance long-term prognostic predictive value for these biomarkers across the injury severity spectrum.

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Manuscript Region of Origin:	UNITED STATES
Abstract:	Objective: Fatigue is a complex, multidimensional phenomenon that commonly occurs following traumatic brain injury (TBI). The thalamus—a structure vulnerable to both primary and secondary injury in TBI—is thought to play a pivotal role in the manifestation of fatigue. We explored how neuroimaging markers of local and global thalamic morphometry relate to the subjective experience of fatigue post-TBI. Methods: 63 Veterans with history of mild TBI (mTBI) underwent structural magnetic resonance scanning (MRI) and completed questionnaires related to fatigue and psychiatric symptomatology. FMRIB's Software (FSL) was utilized to obtain whole brain and thalamic volume estimates, as well as to perform regional thalamic morphometry analyses. Results: Independent of age, sex, intracranial volume, PTSD and depressive symptoms, greater levels of self-reported fatigue was significantly associated with decreased right ($p = .026$) and left ($p = .046$) thalamic volumes. Regional morphometry analyses revealed fatigue was significantly associated with reductions in the anterior and dorsomedial aspects of the right thalamic body ($p < .05$). Similar trends were observed for the left thalamic body ($p < .10$). Conclusions: Both global and regional thalamic morphometry is associated with fatigue in Veterans with history of mTBI. These findings support a theory in which disruption of thalamo-cortico-striatal circuitry

	is thought to result in the manifestation of fatigue. Volumetric and quantified MRI may prove to be a useful tool for elucidating neural structures involved in the subjective experience of fatigue post-injury.
Response to Reviewers:	Reviewer Comments: Reviewer #1: This is a well-written manuscript that aims to understand thalamic structure relationships with self-reported fatigue among a cohort of Veterans with mTBI. Though previous studies exist examining functional connectivity have assessed thalamic atrophy in moderate to severe TBI, and its relationship to executive function and disability, less is known about the impact of thalamic atrophy on fatigue after TBI or prevalence of thalamic atrophy among mTBI. Despite several strengths of the study, including sample size, subregional thalamic analyses, detail provided for imaging methods, mTBI population, neuroanatomical correlates with behavioral assessment, there are issues that should be addressed to further improve this report. Among them include 1) clarity of methodological details about inclusion of individuals with multiple TBI, detailing of window of time since injury, 2) lack of additional imaging analyses in striatum and cortical regions consistent with the hypothesized neural network associated with central fatigue, 3) more explanation should be provided in the statistical section specifically about multivariable "blocks" described in the results as well as adjustment for multiple comparisons and "variance of tolerance tests", 4) operationalization in the methods for definition of "most significant TBI". 1. We agree with the reviewer that additional methodological details regarding TBI history and injury characteristics are critical for the readers to better understand the sample, potential factors of influence in these findings, and the degree to which these findings may generalize to other TBI samples. As such, we have added some additional details regarding the TBI history interview, the collection of critical diagnostic information, and clarified how some of our TBI variables (i.e., total number of TBIs, most significant TBI, cumulative blast-exposure, and time since most recent and most significant TBI) are operationalized. Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime. The vast majority of these additional injuries involved an AOC (82%) versus an LOC (18%). As stated in the methods section, we used the VA/DoD guidelines for diagnosing mTBI, and a notable strength of our study is that we comprehensively assess injuries that occurred before, during, or after service and apply these criteria to characterizing injuries sustained across the lifetime. Please see page 6, lines 132-153, which reads: “Each participant underwent a TBI history interview that was conducted by a post-baccalaureate or graduate-level research assistant. TBI history interviews included assessment of both non-military (i.e., prior to or after discharge from the military) and military-related (i.e., during enlistment in the US armed services) head injuries (see 31 for more comprehensive details). This interview was adapted from the VA Structured Clinical Interview for TBI and involves assessment of any injuries in which participants may have fallen, suffered a blow to the head, or experienced a blast-wave detonation (i.e., overpressure or shock waves resulting from an explosive detonation) within 100 meters (i.e., length of a professional football field). In brief, for each reported injury, participants were queried about: presence and duration of critical injury severity charactertistics (i.e., LOC, AOC, and PTA), the nature of the accident (e.g, fight or motor vechicle accient), the date the injury occurred, the mechanism of injury (i.e., blunt/mechanical or blast-related forces), and the presence and duration of any post-concussive symptoms. Diagnosis of mTBI was based on the VA/DoD guidelines 30 of a loss of consciousness (LOC) < 30 minutes, or alteration of consciousness (AOC) or posttraumatic amnesia (PTA) < 24 hours. The sum of all injuries that met VA/DoD diagnostic criteria for mTBI was calculated to determine the total number of lifetime TBIs each participant experienced. Additionally, injury severity characteristics (i.e., LOC, AOC, PTA) were also utilized to determine the “most significant” or “worst” TBI; this involved direct comparisons of LOC and AOC durations for each injury, and injuries where a LOC was sustained were considered more significant/severe than those that had an AOC only. Finally, the time between participants’ most recent and significant TBI and their neuropsychological testing date was calculated.” Please see page 10, lines 230-236, which reads: “Sample characteristics are presented in Table 1. On average, participants were

relatively young (mean age = 31.9), predominantly male (87%), experienced multiple mTBIs (78%), and were many months removed from their most recent injury (mean time = 63.7 months). With respect to their most significant TBI, 58% of these injuries occurred during deployment, 62% involved a LOC versus an AOC, and 67% were due primarily to blunt-force trauma. Of the individuals with a history of multiple TBIs, approximately 18% reported another injury that involved an LOC versus 82% involved an AOC."

Finally, we have also added the following information to the results section and Table 1: the proportion of individuals with history of one versus multiple TBIs; the median # of TBIs; and mean and median time since most significant TBI.

Structure/Dependent VariableMFIS as a predictor
Left Putamenp = .174
Right Putamenp =.471
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2.The reviewer made an important point that the underlying network of fatigue is relatively broad, and likely involves other cortical and subcortical structures. For the purposes of this study, we took an a-priori, focused approach of investigating thalamic morphometry and fatigue. We chose to focus on thalamus given (1) this structure's demonstrated vulnerability to injury in TBI, (2) the imaging methods we utilized (i.e., thalamic morphometry) have been demonstrated to most reliable in the thalamus (Patenade et al., 2011), and (3) robust and detailed thalamic atlases exists that allow us to more comprehensively understand which nuclei and projections may play a pivotal role in the subjective experience of fatigue post-TBI. This last point is especially critical given that the thalamus is a critical relay station for a wide range of behavioral functions, and no former study has conducted such an exploration in mTBI. Nevertheless, we have conducted another set of analyses to explore potential associations between fatigue and volume and morphometry of the caudate, putamen, nucleus accumbens, and globus pallidus. Parallel regression analyses using the same models revealed no significant associations between fatigue and volume of any of these structures.

Parallel morphometric analyses conducted using FSL's tfce correction revealed no significant associations between fatigue and morphometry of the putamen, nucleus accumbens, globus pallidus, and right caudate. However, there were several small clusters of significant deformation in the head and medial surface of the left caudate.

For purposes of clarity, we have chosen to include this information in the discussion on page 14, lines 316-334:
"Although we chose to focus on the role both global and regional thalamic morphometry have in the role of subjective fatigue post-mTBI, it is important to acknowledge that the underlying network of fatigue is broad and other basal ganglia structures have been also implicated. 4,17,50 Therefore, we conducted a series of

post-hoc analyses to explore potential associations between fatigue and global volumetric and regional morphometry of the caudate, putamen, nucleus accumbens, and globus pallidus using FSL's FIRST output. Parallel statistical models controlling for age, ICV, sex, PCL-M total score, and the BDI Affective subscale total revealed no significant associations between fatigue and lateralized volumes of the caudate, putamen, nucleus accumbens, and globus pallidus (p's ranged from .120 - .567). No significant associations between fatigue and regional morphometry of lateralized putamen, nucleus accumbens, globus pallidus, and the right caudate (all p's >.05) were observed. However, higher levels of fatigue were significantly associated with localized reductions in the head and medial surface of left caudate (p <. 05). The caudate is part of the ventral striatum, receiving projections from the frontal lobe, and also plays a contributory role in effort-reward valuations. Importantly, studies in TBI and other clinical populations have revealed that structural17,53 and functional alterations54-56 to the caudate have been associated with fatigue. While the mechanisms underlying caudate dysfunction are unclear, it, too, may be susceptible to direct trauma or the same negative neuroinflammatory processes. Alternatively, damage to the frontal or basal ganglia dopaminergic system may negatively alter caudate response.57

3.The reviewer asked for additional clarification regarding our statistical analysis plan. First, we did not correct for multiple comparisons in our volumetric analyses given that we focused on an a-priori region of interest (i.e., the thalamus). Given our focused approach, a family-wise alpha of .05 is appropriate, and therefore adjusting for lateralized comparisons is not necessary (see O'Keefe et al., 2003; and Mastunaga, 2007). However, with respect to our morphometry analyses, we used FSL's GLM to conduct 5,000 permutations and used cluster-based thresholding (tfce) that was corrected for multiple comparisons. This method uses family-wise error corrected means in which only p values less than .05 are accepted, is generally more sensitive to finding true signal than voxel-wise thresholding, and produces an output image of significant clusters of spatially similar voxels (see Smith & Nickols, 2009).
With respect to our regression analyses, we were interested in the independent effects of fatigue on thalamic volume/morphometry. However, since age, ICV, and gender are all highly correlated with our dependent variable coupled with overlap between fatigue (i.e., our main independent variable of interest) and PTSD/depressive symptoms, we have chosen to include age, ICV, gender, PTSD, and BDI affective subscale as covariates in our regression models.

For increased clarity, we have added the following text to the statistical analysis section on page 9, lines 213-221 and denoted how multicollinearity was assessed:

"Hierarchical linear regressions were used to determine whether fatigue was predictive of thalamic volumes above and beyond age, sex, ICV, PCL-M and BDI-II Affective subscale scores using the Statistical Package for the Social Sciences (SPSS) version 21. 45 We entered age, ICV, and sex into Block 1 of our regression models given their known associations with brain volume estimates. The PCL-M and BDI-II Affective subscale were entered into Block 1 given our interest in the effects of fatigue—independent of other common psychiatric comorbidities that have been shown across several studies to have some symptom overlap—on thalamic volumes. Multicollinearity of the independent variables was assessed and all variance inflation factor (VIF) values were less than 3.

4. Please see response # 1 to the review's point regarding how most significant TBI was operationalized in this sample.

Reviewer #1: Chief among these issues is the fact that the authors propose a network hypothesis as what underlies central fatigue and the untapped potential to increase the impact of the study by expanding the regional analyses to include striatum and cortex for more of a "network" analysis. Volumetric studies, possibly along with concurrent DTI involved in thalamic projection sub-regions (see PMID: 25862940; PMID: 17296985; PMID: 28365490 as potentially relevant literature), could provide more direct support for the network hypothesis. While depression/PTSD did not correlate with thalamic morphometry, it may be possible that it is linked to morphometry with other regions involved in the proposed circuit hypothesis (see PMID: 28295837 and

PMID: 25184336 and PMID: 24139810 as potentially supportive literature in this regard).

5. We thank the reviewer for this important point and have referenced some of the recommended literature. First, we have expanded our volumetric and morphometry analyses to encompass other subcortical structures (please refer to response #2 above). Secondly, we believe that our findings nicely build upon our prior published DTI work showing that fronto-thalamic projections (i.e., the anterior internal capsule) are significantly associated with self-reported fatigue in Veterans with history of mTBI (see Clark et al., 2016). Indeed, these particular results are what lead us to directly explore both thalamic volumes and morphometry (a more localized metric of atrophy) in the current study. Although we did not directly investigate DTI metrics of the thalamus itself, in reviewing the suggested literature, we have found some additional support for how the findings of the current paper align well with our previous findings. For example, several studies have shown that reduced thalamic volume is significantly associated with decreased thalamic white matter microstructure, especially in regions with fronto-cortico projections. Additionally, the reviewer made an important point with respect to the fact that PTSD/depression may be significantly associated with volume and morphometry of other structures involved in this posited thalamo-cortico-striato loop. Interestingly, simple bivariate correlations revealed no significant associations between volumes and PTSD/depressive symptoms. Moreover, when we explored each individual regression model, neither the PCL-M total score or BDI-II affective scale were significant predictors of volume in Blocks 1 or 2 of the models. Regional morphometry analyses also did not reveal any significant associations with psychiatric symptoms. Nevertheless, we think this information should be presented to the readers, and thus we have added the following text (below). This information is critical to clarifying the nature and underlying cause of fatigue in Veterans with mTBI and co-morbid psychiatric distress.

We have added the following text to further link the significance of our previous findings with the current study's conclusions on page 13, lines 302-304:
Moreover, in studies of aging, lower thalamic volume has been shown to be significantly associated with decreased thalamic white matter microstructure,57,58 especially in regions with fronto-cortico projections.59

Please see the underlined text on page 15, lines 335-345:
"Increased rates of psychiatric symptoms are also common post-TBI 2,7,61 and, while distinct, there is considerable overlap between the clinical symptoms of depression, PTSD, and fatigue. 7,8,62 Additionally, structural and functional alterations in the fronto-limbic and fronto-striatal structures have been linked to increased psychiatric symptom severity post-TBI.63,64 Our results showed that, independent of PTSD and depressive symptoms, fatigue was associated with both global and regional thalamic morphometry. Importantly, neither PTSD nor depressive symptoms were significant predictors of thalamic morphometry in our regression models. Moreover, parallel analyses conducted in the putamen, nucleus accumbens, globus pallidus, and caudate revealed no significant associations between volume, morphometry, and psychiatric symptoms. These results therefore suggest that neurostructural alterations to the thalamus (and caudate) are specifically linked to fatigue post-TBI and not psychiatric symptoms in general. Importantly, these findings help assist in clarifying the nature and underlying cause of specific post-concussive symptoms in those with history of mild TBI.

Reviewer #1: Post hoc analyses regarding thalamic structure/fatigue relationships among those with one vs. multiple TBI might be informative as this VA sample likely has a substantial cohort with repetitive trauma that may reflect different structure-function relationships.

6. As the reviewer noted, there is considerable discussion in the literature about whether brain and behavioral consequences differ in those with single versus repetitive head-trauma. Unfortunately, in the current study, teasing apart differential associations between single vs. multiple TBIs is somewhat challenging given that only a small proportion of the sample reported experiencing a single TBI (i.e., only 22%) during their lifetime. Despite this, however, we attempted to consider the potential influence of total number of TBIs (as well as cumulative blast exposure and time since injury) on our

thalamic volume and morphometry analyses. We added the following text on page 15, lines 348-362:
“Within the mTBI literature, there is some evidence to suggest that negative brain or behavior consequences differ as a function of number of mTBIs, time since injury, or cumulative exposure to blast.66-69 Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime, with the vast majority of these additional injuries involving an AOC versus an LOC. Moreover, there was considerable variability across the sample in time since their most recent mTBIs and whether they were exposed to blast while on deployment. Teasing apart differential associations between single vs. multiple TBIs within the context of this study is challenging given that only a small proportion of the sample reported experiencing only one TBI (i.e., only 22%) during their lifetime. However, we conducted a series of secondary analyses in which total number of TBIs, time since most recent TBI, and total number of blast exposures within 100 meters were included as separate covariates in our regression analyses; results revealed that none of these variables were significantly associated with our dependent variables, and all significant fatigue, thalamic volume and morphometry findings held.”

Reviewer #2: The authors related a measure of fatigue to MRI-determined thalamic volume among in 63 predominantly male Veterans about 5 years after their last TBI. After adjustment for age, sex, intracranial volume, PTSD and depression, fatigue was found to be significantly correlated with R and L thalamic volumes, however the coefficients of variance were small (6.8-8.6%). Most of the variance in thalamic volume was accounted for by the non-fatigue variables, and still those accounted for only 29-30% of the R2.

This is a well-written study with sound methods. But I think the authors' conclusions may have overstated the results. While I would agree that their findings "support a theory in which disruption of thalamo-cortico-striatal circuitry is thought to result in the manifestation of fatigue", I'm not sure that an R2 of 8% would be high enough for volumetric and quantified MRI to be considered " a useful tool for elucidating neural structures involved in the subjective experience of fatigue post-injury".

1. We appreciate reviewer’s points and agree that tempering the language surrounding the utility of structural MRI in examining subjective fatigue is warranted. We have therefore removed this sentence from the abstract and edited a similar sentence in the discussion to read:
“Importantly, these findings implicate the thalamus as a critical structure involved in the underlying neural network of fatigue.”

While the coefficients of variance for fatigue were small (6.8-8.6%), we believe this effect is important, especially since we carefully controlled for so many important covariates and potential confounds. It's often difficult to find brain-behavior associations using MRI, and we think this finding is important, especially since our sample comprises folks that have sustained only mild neurotrauma and traditional MRI metrics have oftentimes been insensitive to mTBI-related brain changes (see Bigler 2013 for a comprehensive review).

	Reviewer #2: I would also ask the authors to comment on the following: Is it possible that the correlation of thalamic volume with fatigue is affected by the number of TBIs, the number of blast exposures, or the time between last TBI and MRI? 2. The reviewer's point that there may be other potential factors of influence on the observed findings is valid. As such, as stated above (please see comment 6), we explored the potential independent effects of these variables in a series of additional analyses and added the following text to the manuscript on We added the following text on page 15, lines 348-362: "Within the mTBI literature, there is some evidence to suggest that negative brain or behavior consequences differ as a function of type of injury or number of mTBIs, time since injury, or cumulative exposure to blast.66-69 Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime, with the vast majority of these additional injuries involving an AOC versus an LOC. Moreover, there was considerable variability across the sample in time since their most recent mTBIs and whether they were exposed to blast while on deployment. Teasing apart differential associations between single vs. multiple TBIs within the context of this study is challenging given that only a small proportion of the sample reported experiencing a single TBI (i.e., only 22%) during their lifetime. However, we conducted a series of secondary analyses in which total number of TBIs, time since most recent TBI, and total number of blast exposures within 100 meters were included as separate covariates in our regression analyses; results revealed that none of these variables were significantly associated with our dependent variables, and all significant fatigue, thalamic volume and morphometry findings held."

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November 2, 2017

John D. Corrigan, Ph.D, ABPP
Editor-in-Chief, *Journal of Head Trauma Rehabilitation*
Director, Division of Rehabilitation Psychology
Professor, Department of Physical Medicine and Rehabilitation
Ohio State University

Dear Dr. Corrigan,

We would like to thank both you and the reviewers for the thoughtful critique of our manuscript entitled *"Fatigue is Associated with Global and Regional Thalamic Morphometry in Veterans with History of Mild Traumatic Brain Injury."* We believe that the comments and suggestions made by reviewers have further strengthened and focused the manuscript, and we are now resubmitting it for further consideration for publication in your esteemed journal, *Journal of Head Trauma Rehabilitation*.

To our knowledge, this is the first study to explore how global and regional thalamic morphometry relates to fatigue in Veterans with history of mild TBI. Although several structural MRI studies have been conducted in TBI over the past several years, the present study significantly expands upon the existing literature by directly relating structural MRI techniques to a common, chronic, and troubling post-concussive symptom (i.e., fatigue) following TBI. Moreover, this work extends our understanding of the underlying neural substrates of behaviorally manifested fatigue by directly implicating the thalamus.

As you noted, the initial reviews to our manuscript were generally favorable, although there were some concerns regarding (1) the characterization of the participant's exposure to TBI and (2) the rationale for limiting investigation to the thalamus. We have addressed these critiques by including additional methodological details regarding assessment of TBI history and injury characteristics, and we have better clarified how TBI variables were operationalized within the study. Additionally, we have expanded our analyses to include other subcortical regions that have been implicated in the subjective experience of fatigue.

Below, we summarize the reviewer's comments (black ink) along with our responses (blue ink), and we underline where the changes were made within the manuscript. As stated in the original submission, these findings have not been submitted elsewhere for publication, and all appropriate IRB assurances were in place for this study.

If you have any questions or require any additional information, please do not hesitate to contact me by phone (858-552-8585 ext. 2664) or email: ldelano@ucsd.edu Thank you for your consideration and I look forward to your correspondence.

Lisa Delano-Wood, Ph.D.

Associate Professor, Department of Psychiatry, UCSD Medical School
Staff Psychologist, VA San Diego Healthcare System, (858) 552-8585 x2667

Sep 23, 2017

RE: JHTR-D-17-00113, entitled "Fatigue is Associated with Global and Regional Thalamic Morphometry in Veterans with History of Mild Traumatic Brain Injury"

Dear Ms. Clark,

I am pleased to inform you that the reviews of the above paper submitted to the Journal of Head Trauma Rehabilitation were generally favorable. However, the reviewers have posed specific questions that must be addressed before publication. Of particular concern is the characterization of the participant's exposure to TBI as well as the rationale for limiting investigation to the thalamus. Please find all comments of both reviewers below.

If you are willing to revise the manuscript taking into consideration the suggestions of the reviewers, I will send the revised paper to the original reviewers for re-appraisal. Please include with your revised submission an itemized, point-by-point response to all comments of the reviewers. Denote changes in the manuscript using either tracked changes or by highlighting new text. The revisions should be completed by Oct 23, 2017 to avoid being considered as a new submission. If you do not submit a revision, the manuscript will be withdrawn by the editorial staff.

To submit a revision, go to http://jhtr.edmgr.com/ and log in as an Author. You will see a menu item called "Submission Needing Revision." Please click on this item to obtain your submission record and begin the revision process.

Your username is: ********
****

With Kind Regards,

John D. Corrigan, PhD
Editor-in-Chief
Journal of Head Trauma Rehabilitation

Reviewer Comments:

Reviewer #1: This is a well-written manuscript that aims to understand thalamic structure relationships with self-reported fatigue among a cohort of Veterans with mTBI. Though previous studies exist examining functional connectivity have assessed thalamic atrophy in moderate to severe TBI, and its relationship to executive function and disability, less is known about the impact of thalamic atrophy on fatigue after TBI or prevalence of thalamic atrophy among mTBI. Despite several strengths of the study, including sample size, subregional thalamic analyses, detail provided for imaging methods, mTBI population, neuroanatomical correlates with behavioral assessment, there are issues that should be addressed to further improve this report. Among them include 1) clarity of methodological details about inclusion of individuals with multiple TBI, detailing of window of time since injury, 2) lack of additional imaging analyses in striatum and cortical regions consistent with the hypothesized neural network associated with central fatigue, 3) more explanation should be provided in the statistical section specifically about multivariable "blocks" described in the results as well as adjustment for multiple comparisons and "variance of tolerance tests", 4) operationalization in the methods for definition of "most significant TBI".

1. We agree with the reviewer that additional methodological details regarding TBI history and injury characteristics are critical for the readers to better understand the sample, potential factors of influence in these findings, and the degree to which these findings may generalize to other TBI samples. As such, we have added some additional details regarding the TBI history interview, the collection of critical diagnostic information, and clarified how some of our TBI variables (i.e., total number of TBIs, most significant TBI, cumulative blast-exposure, and time since most recent and most significant TBI) are operationalized. Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime. The vast majority of these additional injuries involved an AOC (82%) versus an LOC (18%). As stated in the methods section, we used the VA/DoD guidelines for diagnosing mTBI, and a notable strength of our study is that we comprehensively assess injuries that occurred before, during, or after service and apply these criteria to characterizing injuries sustained across the lifetime.

Please see page 6, lines 132-153, which reads:

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Finally, we have also added the following information to the results section and Table 1: the proportion of individuals with history of one versus multiple TBIs; the median # of TBIs; and mean and median time since most significant TBI.

2. The reviewer made an important point that the underlying network of fatigue is relatively broad, and likely involves other cortical and subcortical structures. For the purposes of this study, we took an a-priori, focused approach of investigating thalamic morphometry and fatigue. We chose to focus on thalamus given (1) this structure's demonstrated vulnerability to injury in TBI, (2) the imaging methods we utilized (i.e., thalamic morphometry) have been demonstrated to most reliable in the thalamus (Patenade et al., 2011), and (3) robust and detailed thalamic atlases exists that allow us to more comprehensively understand which nuclei and projections may play a pivotal role in the subjective experience of fatigue post-TBI. This last point is especially critical given that the thalamus is a critical relay station for a wide range of behavioral functions, and no former study has conducted such an exploration in mTBI. Nevertheless, we have conducted another set of analyses to explore potential associations between fatigue and volume and morphometry of the caudate, putamen, nucleus accumbens, and globus pallidus. Parallel regression analyses using the same models revealed no significant associations between fatigue and volume of any of these structures.

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"Although we chose to focus on the role both global and regional thalamic morphometry have in the role of subjective fatigue post-mTBI, it is important to acknowledge that the underlying network of fatigue is broad and other basal ganglia structures have been also implicated. [4,17,50] Therefore, we conducted a series of post-hoc analyses to explore potential associations between fatigue and global volumetric and regional morphometry of the caudate, putamen, nucleus accumbens, and globus pallidus using FSL's FIRST output. Parallel statistical models controlling for age, ICV, sex, PCL-M total score, and the BDI Affective subscale total revealed no significant associations between fatigue and lateralized volumes of the caudate, putamen, nucleus accumbens, and globus pallidus (*p*'s ranged from .120 - .567). No significant associations between fatigue and regional morphometry of lateralized putamen, nucleus accumbens, globus pallidus, and the right caudate (all *p*'s >.05) were observed. However, higher levels of fatigue were significantly associated with localized reductions in the head and medial surface of left caudate ($p < .05$). The caudate is part of the ventral striatum, receiving projections from the frontal lobe, and also plays a contributory role in effort-reward valuations. Importantly, studies in TBI and other clinical populations have revealed that structural[17,53] and functional alterations[54-56] to the caudate have been associated with fatigue. While the mechanisms underlying caudate dysfunction are unclear, it, too, may be susceptible to direct trauma or the same negative neuroinflammatory processes. Alternatively, damage to the frontal or basal ganglia dopaminergic system may negatively alter caudate response.[57]

3. The reviewer asked for additional clarification regarding our statistical analysis plan. First, we did not correct for multiple comparisons in our volumetric analyses given that we focused on an a-priori region of interest (i.e., the thalamus). Given our focused approach, a family-wise alpha of .05 is appropriate, and therefore adjusting for lateralized comparisons is not necessary (see O'Keefe et al., 2003; and Mastunaga, 2007). However, with respect to our morphometry analyses, we used FSL's GLM to conduct 5,000 permutations and used cluster-based thresholding (tfce) that was corrected for multiple comparisons. This method uses family-wise error corrected means in which only p values less than .05 are accepted, is generally more sensitive to finding true signal than voxel-wise thresholding, and produces an output image of significant clusters of spatially similar voxels (see Smith & Nickols, 2009).

   With respect to our regression analyses, we were interested in the *independent effects* of fatigue on thalamic volume/morphometry. However, since age, ICV, and gender are all highly correlated with our dependent variable coupled with overlap between fatigue (i.e., our main independent variable of interest) and PTSD/depressive symptoms, we have chosen to include age, ICV, gender, PTSD, and BDI affective subscale as covariates in our regression models.

For increased clarity, we have added the following text to the statistical analysis section on page 9, lines 213-221 and denoted how multicollinearity was assessed:

"Hierarchical linear regressions were used to determine whether fatigue was predictive of thalamic volumes above and beyond age, sex, ICV, PCL-M and BDI-II Affective subscale scores using the Statistical Package for the Social Sciences (SPSS) version 21. [45] We entered age, ICV, and sex into Block 1 of our regression models given their known associations with brain volume estimates. The PCL-M and BDI-II Affective subscale were entered into Block 1 given our interest in the effects of fatigue—independent of other common psychiatric comorbidities that have been shown across several studies to have some symptom overlap—on thalamic volumes. Multicollinearity of the independent variables was assessed and all variance inflation factor (VIF) values were less than 3.

4. Please see response # 1 to the review's point regarding how most significant TBI was operationalized in this sample.

Reviewer #1: Chief among these issues is the fact that the authors propose a network hypothesis as what underlies central fatigue and the untapped potential to increase the impact of the study by expanding the regional analyses to include striatum and cortex for more of a "network" analysis. Volumetric studies, possibly along with concurrent DTI involved in thalamic projection sub-regions (see PMID: 25862940; PMID: 17296985; PMID: 28365490 as potentially relevant literature), could provide more direct support for the network hypothesis. While depression/PTSD did not correlate with thalamic morphometry, it may be possible that it is linked to morphometry with other regions involved in the proposed circuit hypothesis (see PMID: 28295837 and PMID: 25184336 and PMID: 24139810 as potentially supportive literature in this regard).

5. We thank the reviewer for this important point and have referenced some of the recommended literature. First, we have expanded our volumetric and morphometry analyses to encompass other subcortical structures (please refer to response #2 above). Secondly, we believe that our findings nicely build upon our prior published DTI work showing that fronto-thalamic projections (i.e., the anterior internal capsule) are significantly associated with self-reported fatigue in Veterans with history of mTBI (see Clark et al., 2016). Indeed, these particular results are what lead us to directly explore both thalamic volumes and morphometry (a more localized metric of atrophy) in the current study. Although we did not directly investigate DTI metrics of the thalamus itself, in reviewing the suggested literature, we have found some additional support for how the findings of the current paper align well with our previous findings. For example, several studies have shown that reduced thalamic volume is significantly associated with decreased thalamic white matter microstructure, especially in regions with fronto-cortico projections. Additionally, the reviewer made an important point with respect to the fact that PTSD/depression may be significantly associated with volume and morphometry of other structures involved in this posited thalamo-cortico-striato loop. Interestingly, simple bivariate correlations revealed no significant associations between volumes and PTSD/depressive symptoms. Moreover, when we explored each individual regression model, neither the PCL-M total score or BDI-II affective scale were significant predictors of volume in Blocks 1 or 2 of the models. Regional morphometry analyses also did not reveal any significant associations with psychiatric symptoms. Nevertheless, we think this information should be presented to the readers, and thus we have added the following text (below). This information is critical to clarifying the nature and underlying cause of fatigue in Veterans with mTBI and co-morbid psychiatric distress.

We have added the following text to further link the significance of our previous findings with the current study's conclusions on page 13, lines 302-304:

> Moreover, in studies of aging, lower thalamic volume has been shown to be significantly associated with decreased thalamic white matter microstructure,[57,58] especially in regions with fronto-cortico projections.[59]

Please see the underlined text on page 15, lines 335-345:

> "Increased rates of psychiatric symptoms are also common post-TBI [2,7,61] and, while distinct, there is considerable overlap between the clinical symptoms of depression, PTSD, and fatigue. [7,8,62] Additionally, structural and functional alterations in the fronto-limbic and fronto-striatal structures have been linked to increased psychiatric symptom severity post-TBI.[63,64] Our results showed that, independent of PTSD and depressive symptoms, fatigue was associated with both global and regional thalamic morphometry. Importantly, neither PTSD nor depressive symptoms were significant predictors of thalamic morphometry in our regression models. Moreover, parallel analyses conducted in the putamen, nucleus accumbens, globus pallidus, and caudate revealed no significant associations between volume, morphometry, and psychiatric symptoms. These results therefore suggest that neurostructural alterations to the thalamus (and caudate) are specifically linked to fatigue

post-TBI and not psychiatric symptoms in general. Importantly, these findings help assist in clarifying the nature and underlying cause of specific post-concussive symptoms in those with history of mild TBI.

Reviewer #1: Post hoc analyses regarding thalamic structure/fatigue relationships among those with one vs. multiple TBI might be informative as this VA sample likely has a substantial cohort with repetitive trauma that may reflect different structure-function relationships.

6. As the reviewer noted, there is considerable discussion in the literature about whether brain and behavioral consequences differ in those with single versus repetitive head-trauma. Unfortunately, in the current study, teasing apart differential associations between single vs. multiple TBIs is somewhat challenging given that only a small proportion of the sample reported experiencing a single TBI (i.e., only 22%) during their lifetime. Despite this, however, we attempted to consider the potential influence of total number of TBIs (as well as cumulative blast exposure and time since injury) on our thalamic volume and morphometry analyses. We added the following text on page 15, lines 348-362:

> "Within the mTBI literature, there is some evidence to suggest that negative brain or behavior consequences differ as a function of number of mTBIs, time since injury, or cumulative exposure to blast.[66-69] Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime, with the vast majority of these additional injuries involving an AOC versus an LOC. Moreover, there was considerable variability across the sample in time since their most recent mTBIs and whether they were exposed to blast while on deployment. Teasing apart differential associations between single vs. multiple TBIs within the context of this study is challenging given that only a small proportion of the sample reported experiencing only one TBI (i.e., only 22%) during their lifetime. However, we conducted a series of secondary analyses in which total number of TBIs, time since most recent TBI, and total number of blast exposures within 100 meters were included as separate covariates in our regression analyses; results revealed that none of these variables were significantly associated with our dependent variables, and all significant fatigue, thalamic volume and morphometry findings held."

Reviewer #2: The authors related a measure of fatigue to MRI-determined thalamic volume among in 63 predominantly male Veterans about 5 years after their last TBI. After adjustment for age, sex, intracranial volume, PTSD and depression, fatigue was found to be significantly correlated with R and L thalamic volumes, however the coefficients of variance were small (6.8-8.6%). Most of the variance in thalamic volume was accounted for by the non-fatigue variables, and still those accounted for only 29- 30% of the R2.

This is a well-written study with sound methods. But I think the authors' conclusions may have overstated the results. While I would agree that their findings "support a theory in which disruption of thalamo-cortico-striatal circuitry is thought to result in the manifestation of fatigue", I'm not sure that an R2 of 8% would be high enough for volumetric and quantified MRI to be considered " a useful tool for elucidating neural structures involved in the subjective experience of fatigue post-injury".

1. We appreciate reviewer's points and agree that tempering the language surrounding the utility of structural MRI in examining subjective fatigue is warranted. We have therefore removed this sentence from the abstract and edited a similar sentence in the discussion to read:

> "Importantly, these findings implicate the thalamus as a critical structure involved in the underlying neural network of fatigue."

While the coefficients of variance for fatigue were small (6.8-8.6%), we believe this effect is important, especially since we carefully controlled for so many important covariates and potential confounds. It's often difficult to find brain-behavior associations using MRI, and we think this finding is important, especially since our sample comprises folks that have sustained only *mild* neurotrauma and traditional MRI metrics have oftentimes been insensitive to mTBI-related brain changes (see Bigler 2013 for a comprehensive review).

Reviewer #2: I would also ask the authors to comment on the following: Is it possible that the correlation of thalamic volume with fatigue is affected by the number of TBIs, the number of blast exposures, or the time between last TBI and MRI?

2. The reviewer's point that there may be other potential factors of influence on the observed findings is valid. As such, as stated above (please see comment 6), we explored the potential independent effects of these variables in a series of additional analyses and added the following text to the manuscript on We added the following text on page 15, lines 348-362:

"Within the mTBI literature, there is some evidence to suggest that negative brain or behavior consequences differ as a function of type of injury or number of mTBIs, time since injury, or cumulative exposure to blast.[66-69] Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime, with the vast majority of these additional injuries involving an AOC versus an LOC. Moreover, there was considerable variability across the sample in time since their most recent mTBIs and whether they were exposed to blast while on deployment. Teasing apart differential associations between single vs. multiple TBIs within the context of this study is challenging given that only a small proportion of the sample reported experiencing a single TBI (i.e., only 22%) during their lifetime. However, we conducted a series of secondary analyses in which total number of TBIs, time since most recent TBI, and total number of blast exposures within 100 meters were included as separate covariates in our regression analyses; results revealed that none of these variables were significantly associated with our dependent variables, and all significant fatigue, thalamic volume and morphometry findings held."

Fatigue is Associated with Global and Regional Thalamic Morphometry in Veterans with History of Mild Traumatic Brain Injury

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Compliance with Ethical Standards & Disclosures. All procedures involved in this study were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975. Informed consent was obtained from all patients included in the study. Alexandra Clark, Scott Sorg, Erin Bigler, Kelsey Holiday, Katherine Bangen, Nicole Evangelista, Mark Bondi, Dawn Schiehser, and Lisa Delano-Wood declare no conflicts of interest.

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ABSTRACT

Objective: Fatigue is a complex, multidimensional phenomenon that commonly occurs following traumatic brain injury (TBI). The thalamus—a structure vulnerable to both primary and secondary injury in TBI—is thought to play a pivotal role in the manifestation of fatigue. We explored how neuroimaging markers of local and global thalamic morphometry relate to the subjective experience of fatigue post-TBI. **Methods:** 63 Veterans with history of mild TBI (mTBI) underwent structural magnetic resonance scanning (MRI) and completed questionnaires related to fatigue and psychiatric symptomatology. FMRIB's Software (FSL) was utilized to obtain whole brain and thalamic volume estimates, as well as to perform regional thalamic morphometry analyses. **Results:** Independent of age, sex, intracranial volume, PTSD and depressive symptoms, greater levels of self-reported fatigue was significantly associated with decreased right ($p = .026$) and left ($p = .046$) thalamic volumes. Regional morphometry analyses revealed that fatigue was significantly associated with reductions in the anterior and dorsomedial aspects of the right thalamic body ($p < .05$). Similar trends were observed for the left thalamic body ($p < .10$). **Conclusions:** Both global and regional thalamic morphometric changes are associated with the subjective experience of fatigue in Veterans with history of mTBI. These findings support a theory in which disruption of thalamo-cortico-striatal circuitry may result in the manifestation of fatigue in individuals with history of neurotrauma.

INTRODUCTION

Central fatigue is a complex, multi-dimensional phenomenon that is common and frequently intractable in the aftermath of traumatic brain injury (TBI). [1-4] It is distinct from more *peripheral* categorizations of fatigue (i.e., disorders of the neuromuscular junction or muscular fatigue) given that it manifests as a result of *central* nervous system damage and/or dysfunction and it has been conceptualized as "the failure to initiate and/or sustain attentional tasks and physical activities requiring self-motivation."[5(p35)] Central fatigue (referred to as *fatigue* here within) has components that are both cognitive and physical [5] and, although it overlaps with psychiatric and sleep disturbances, fatigue has been demonstrated to be a distinct or independent construct. [6-8] Importantly, across all severities of TBI (i.e. mild, moderate, or severe), fatigue has been linked to worse outcomes, including increased levels of disability and poorer quality of life. [9-11]

Although the underlying neural network of fatigue remains to be fully characterized, there is a growing body of literature to suggest that thalamus may play a critical role. The thalamus—a subcortical gray matter structure that contains many groups of nuclei—is a critical relay station for a diffuse network of afferent and efferent projections within the brain. [12-14] It processes both motor and sensory information and contributes to high-order cognitive processes including memory and executive functions. [15,16] The thalamus is part of the ascending reticular activating system (ARAS), which ensures adequate levels of arousal needed to carry out various behavioral tasks [17] and is thus positioned to play a critical role in the manifestation of fatigue. In support of this position, structural changes to the thalamus have been linked to greater levels of fatigue across various clinical populations including multiple sclerosis and chronic fatigue syndrome. [18-20]

Within the context of TBI, the thalamus is susceptible to both primary and secondary mechanisms of injury.[21,22] Simulations from finite element head modeling have revealed the thalamus is a region of high shear stress during neurotrauma. [23,24] Furthermore, secondary neuroinflammatory and degenerative processes—which have been demonstrated to persist for many years following neurotrauma—also contribute to thalamic damage following TBI. [16,25-27] Structural magnetic resonance imaging (MRI) methods have been used to examine both global and more nuanced topographic, or regional, alterations of the thalamus post-TBI. Across samples of moderate to severe TBI, global reductions in thalamic volume and regional atrophy in antero-dorsal-medial aspects of the thalamus have been observed post-injury.[28] Moreover, regional changes in thalamic morphometry has been linked to greater rates of disability [29] and poorer performance on measures of executive control [21,26] in those with history of moderate to severe TBI.

Studies examining both global and regional morphometry in less severe TBI samples (i.e., mild TBI [mTBI]) are relatively limited. Global reductions in thalamic volume [30,31] have been observed and only one study has explored regional morphometry of the thalamus within an mTBI sample. [32] In contrast to the previous studies in moderate to severe TBI samples, regional reductions were limited to the posterolateral portion of the thalamus of the mTBI group relative to orthopedic-injured controls. [32] However, it remains unclear to what degree these alterations in thalamic morphometry may contribute to the subjective experience of fatigue post-mTBI. Therefore, we explored the potential role thalamic integrity may play in the manifestation of fatigue in mTBI. We hypothesized that greater levels of fatigue would be associated with both decreased volume as well as local atrophy in the thalamus in Veterans with history of mTBI.

METHODS

Participants were 63 Operation Enduring Freedom, Operation Iraqi Freedom, and Operation New Dawn (OEF/OIF/OND) Veterans with history of mTBI recruited from posted study advertisements and outpatient clinics at the VA San Diego Healthcare System (VASDHS) in La Jolla, California. The institutional review boards (IRBs) at the VASDHS and University of California, San Diego (UCSD) approved all study procedures. Prior to enrollment in the study, participants provided written and informed consent. TBI history interviews, neuropsychological testing, and study questionnaires were completed at the Veterans Medical Research Foundation located on the VASDHS campus. Brain MRI scans occurred at the UCSD School of Medicine's Center for Functional MRI.

The following exclusionary criteria were applied to the study sample overall: (1) moderate (loss of consciousness [LOC] >30 minutes but < 24 hours, and alteration of consciousness [AOC] > 24 hours or post-traumatic amnesia [PTA] >1 day but < 7 days) or severe (LOC ≥ 24 hours, AOC > 24 hours, or PTA ≥ 7 days) TBI per [33] guidelines; (2) current (within 30 days) alcohol or other substance abuse, (3) current or prior alcohol or substance dependence; (4) current or prior diagnosis of bipolar disorder, schizophrenia, or psychotic disorder as determined by the *Diagnostic and Statistical Manual of Mental Disorders* (4^{th} ed., DSM-IV, American Psychiatric Association, 2000); (5) a positive toxicology screen as measured by the Rapid Response 10-drug Test Panel; (6) prior history of a learning disability; (7) history of any other major neurological (e.g., multiple sclerosis, stroke, seizures) or medical conditions (e.g., chronic fatigue syndrome, diabetes, myocardial infarction) that may affect brain structure or cognition; (8) current suicidal and/or homicidal ideation; (9) involvement in current or pending litigation; (10) any contraindications to MRI scanning (e.g., presence of metal in one's

body, or possible pregnancy for females participants); and (11) any gross abnormalities on structural MRI scans.

TBI History Interview and Diagnosis

Each participant underwent a TBI history interview that was conducted by a post-baccalaureate or graduate-level research assistant. TBI history interviews included assessment of both non-military (i.e., prior to or after discharge from the military) and military-related (i.e., during enlistment in the US armed services) head injuries (see [34] for more comprehensive details). This interview was adapted from the VA Structured Clinical Interview for TBI[35] and involves assessment of any injuries in which participants may have fallen, suffered a blow to the head, or experienced a blast-wave detonation (i.e., overpressure or shock waves resulting from an explosive detonation) within 100 meters (i.e., length of a professional football field). In brief, for each reported injury, participants were queried about: presence and duration of critical injury severity charactertistics (i.e., LOC, AOC, and PTA), the nature of the accident (e.g, fight or motor vechicle accient), the date the injury occurred, the mechanism of injury (i.e., blunt/mechanical or blast-related forces), and the presence and duration of any post-concussive symptoms. Diagnosis of mTBI was based on the VA/DoD guidelines [33] of a loss of consciousness (LOC) < 30 minutes, or alteration of consciousness (AOC) or posttraumatic amnesia (PTA) < 24 hours. The sum of all injuries that met VA/DoD diagnostic criteria for mTBI was calculated to determine the total number of lifetime TBIs each participant experienced. Additionally, injury severity characteristics (i.e., LOC, AOC, PTA) were also utilized to determine the "most significant" or "worst" TBI; this involved direct comparisons of LOC and AOC durations for each injury, and injuries where a LOC was sustained were considered more significant/severe than those that had an AOC only. Finally, the time between

participants' most recent and significant TBI and their neuropsychological testing date was calculated."

Assessment of Fatigue

The Modified Fatigue Impact Scale (MFIS) was used to assess current levels of fatigue and its impact on functioning over the past four weeks. The MFIS is a 21-item scale modified from the original 40-item Fatigue Impact Scale [36] that was recently validated for use in Veterans with history of mild or moderate TBI. [37] Items from the MFIS can be summed to generate a total score (ranging from 0 to 84), with higher scores indicating greater effects of fatigue on both cognitive and physical functions.

Psychiatric Symptom Rating Scales and Other Self-Report Questionnaires

Participants completed symptom rating scales that quantified current levels of posttraumatic stress (PTSD Checklist [PCL-M] [38]), depression (Beck-Depression Inventory-II [BDI-II] [39]), and post-concussive symptoms (Neurobehavioral Symptom Inventory [NSI] [40]). For the BDI-II, an affective subscale [1,41] was also generated for use in subsequent analyses given some items overlap with the characterization of fatigue. Exposure to various combat situations and wartime stressors during deployment was assessed using the Combat Exposure Scale ([CES] [42]). Overall sleep quality for the past month was assessed with the Pittsburgh Sleep Quality Index ([PSQI] [43]).

Neuroimaging Data Acquisition

Structural MRI scans were acquired on a 3-Tesla General Electric MR750 system with an eight-channel head coil. A high-resolution T1 anatomical scan was acquired in the sagittal plane with the following parameters: FOV = 24 cm, 256 x 192 matrix, TR = 8.1 ms, TE = 3.192 ms, flip angle = 12°, TI = 550 ms, bandwidth = 31.25 kHz, and 172 1.2 mm slices. After the

images were acquired, they were visually inspected for quality control purposes to ensure there were no artifacts that might affect image processing (e.g., motion).

Neuroimaging Processing & Analyses

Images were processed and analyzed using FMRIB Software Library version 5.0. [44,45] Thalamic segmentation and shape analysis was performed with FMRIB Software Library's Integrated Registration and Segmentation Tool ([FIRST]; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST) which has been demonstrated to have the highest concordance among subcortical structures when compared to manual segmentation methods. [46] FIRST uses a two-stage affine process to register each participant's T1 onto an MNI152 template. In the first step, a whole brain registration was performed. Next, a weighted subcortical mask was applied to the intermediate image, which then underwent another affine registration to ensure optimal registration of subcortical structures. The inverse transformation of the estimated registration was then calculated and applied to the MNI152 template to bring it into native space. A Bayesian Active Appearance Model (AAM), which takes into account both geometric shape (i.e., morphometry) and image intensity, was used to register and segment subcortical structures.[43] After registration, a parameterized surface mesh for each subcortical structure is generated. FSL's *–useReconNative* and *-useRigidAlign* options were used to recreate the mesh in native space and remove pose effects. Registrations and thalamic segmentations were checked with FSL's *slicesdir* command.

Global and Regional Thalamic Morphometry

Thalamic volume estimates were extracted from the FIRST segmentation. Intracranial volume (ICV) estimation occurred through the FMRIB Software Library [44,45] using ENGIMA imaging protocol guidelines (http://enigma.ini.usc.edu). FIRST also enables examination of

localized morphometric differences in subcortical structures. A deformable surface mesh model composed of sets of vertices is generated for each subject and allows for the mean shape and any deviations from the mean to be explored. As such, regional morphometry analyses examine any expansions and reductions from the TBI group's mean surface level of the thalamic body. Reduced regional morphometry is associated with negative perpendicular distances from the average surface and expansions represent positive perpendicular distances. [46] In our study, we conducted vertex-wise analyses using general linear modeling (GLM) to explore thalamic morphometry and fatigue associations in the sample. The International Consortium for Brain Mapping Atlas (ICBM) T1 Atlas, [47] which parcellates the thalamus into 11 structurally defined regions of interest (ROIs), was then used to provide additional context about thalamic subnuclei likely involved in the observed regional findings. Prior to overlaying the regional thalamic findings to determine the overlapping ROIs, the ICBM Atlas was registered to MNI-152 space using FSL's FMRIB's Linear Image Registration Tool (FLIRT). ICV was calculated by multiplying the size of the template brain by the inverse of the determinant of the affine matrix.

Statistical Analyses

Hierarchical linear regressions were used to determine whether fatigue was predictive of thalamic volumes above and beyond age, sex, ICV, PCL-M and BDI-II Affective subscale scores using the Statistical Package for the Social Sciences (SPSS) version 21. [48] We entered age, ICV, and sex into Block 1 of our regression models given their known association with brain volume estimates. The PCL-M and BDI-II Affective subscale were also entered into Block 1 since we were interested in the effects of fatigue—independent of other common psychiatric comorbidities that have been shown across several studies to have some symptom overlap—

on thalamic volumes. Multicollinearity of the independent variables was assessed and all variance inflation factor (VIF) values were less than 3. FMIRB's GLM tool (www.fmrib.ox.ac.uk/fsl/fsl/list) was used to explore associations between fatigue and thalamic morphometry while controlling for age, ICV, PCL-M and BDI-II Affective subscale scores. For regional thalamic morphometry analyses, FSL's Randomise tool (www.fmrib.ox.ac.uk/fsl/randomise) was used to test correlation inferences with permutation methods. [49,50] Positive and negative associations for thalamic morphometry and fatigue were explored with 5,000 two-tailed Monte Carlo permutation tests with threshold-cluster free enhancement to correct for multiple comparisons at $p = .05$.

RESULTS

Sample characteristics are presented in Table 1. On average, participants were relatively young (mean age = 31.9), predominantly male (87%), experienced multiple mTBIs (78%), and were many months removed from their most recent injury (mean time = 63.7 months). With respect to their most significant TBI, 58% of these injuries occurred during deployment, 62% involved a LOC versus an AOC, and 67% were due primarily to blunt-force trauma. Of the individuals with a history of multiple TBIs, approximately 18% reported another injury that involved an LOC versus 82% involved an AOC.

[INSERT TABLE 1 AND 2 AROUND HERE]

Association Between Fatigue and Thalamic Volume

Hierarchical regressions were performed in an effort to determine whether fatigue was predictive of thalamic volume for each hemisphere. Age, ICV, sex, PCL-M total score, and the BDI-II Affective subscale total were entered into Block 1, and the MFIS total score was entered into Block 2 of the models. Results are presented in Table 2. Block 1 explained 28.6% of the

variance in right thalamic volume. A significant increase in the amount of variance in right thalamic volume was observed when the MFIS total score was added into Block 2 of the model (R^2 Δ = .062, F Δ (1,56) = 5.32, p = .025). Results indicated that lower right thalamic volumes were associated with higher levels of fatigue (see Figure 1). For left thalamic volume, Block 1 explained 30.3% of the variance, which was also increased with the addition of MFIS total score into Block 2 of the model (R^2 Δ = .048, F Δ (1,56) = 4.15, p = .046). Results indicated that lower left thalamic volumes were associated with higher levels of fatigue. See Figure 2.

[INSERT FIGURE 1 AROUND HERE]

Associations Between Fatigue and Regional Thalamic Morphology

To test for an association between fatigue and regional thalamic morphometry, a series of regression analyses were performed with FSL's GLM and *randomise* tool. Significant negative correlations between fatigue and right thalamic morphometry were found, even after adjusting for age, ICV, sex, PCL-M total score, and the BDI-II Affective subscale total. See Figure 3 for the significant clusters. Results were overlaid onto the ICBM Atlas to provide additional context about the thalamic nuclei that correspond to the atrophied areas. In particular, reductions in the anterior and dorso-medial aspects of the right thalamus were associated with higher levels of self-reported fatigue (p's < .05). With respect to the left thalamus, there were no significant associations between morphometry and fatigue that survived multiple comparisons (p's > .05). However, when alpha was adjusted to p < .10 to explore potential trends, associations between higher levels of fatigue and localized reductions in the anterior, dorsomedial, and reticular aspects of the left thalamus were observed. See Figure 4.

[INSERT FIGURE 2 AROUND HERE]

DISCUSSION

Our study provides evidence that the thalamus—a subcortical gray matter nucleus responsible for processing much of our sensory, motor, cognitive and emotional information, and coordinating behavior[51]—is involved in the subjective experience of fatigue following mTBI. In particular, we found that greater levels of fatigue were associated with (1) decreased thalamic volumes and (2) regional reductions in the anterior and dorsomedial aspects of the thalamic body. Importantly, these findings implicate the thalamus as an important structure involved in the underlying neural network of fatigue. Results dovetail with findings from other clinical populations (i.e., multiple sclerosis) demonstrating that the thalamus is involved in the manifestation of fatigue-related symptoms.[18,19,52] Moreover, given careful consideration of important covariates and possible confounds, our results suggest that neurostructural alterations to the thalamus are specifically linked to fatigue post-TBI and not psychiatric symptoms in general.

Chaudhuri and Behan [5] described a model in which disruption and/or dysfunction of the neural circuitry of the thalamo-cortico-striatal loop is thought to result in fatigue. They argue that, when this circuit is functioning normally, there is a positive feedback loop in which: (1) glutamatergic (excitatory) signals from the frontal cortex lead to excitation of the striatum, (2) striatal activation increases the GABAergic (inhibitory) signals of the substantia nigra and globus pallidus, which then (3) decreases the inhibitory signals of the globus pallidus and substantia nigra on the thalamus, resulting in (4) excitatory output from the thalamus to the frontal cortex (see [53] for comprehensive review of this pathway). *Global* thalamic volume loss may thus cause a net change in thalamic activity, and therefore disrupt the behavioral functions associated with these said pathways. Indeed, relative to controls with no history of head-trauma, resting-state

functional MRI has revealed decreased thalamic activity [31] and abnormal thalamo-cortical connectivity patterns in mTBI patients. [14,54]

Results from our study revealed that greater levels of fatigue were associated with reduced *regional* thalamic morphometry in the anterior and dorsomedial body of the thalamus. Regarding neuroanatomical connections, the anterior nucleus projects efferent fibers to the oribitofrontal and cingulate cortices, while the dorsomedial nucleus sends fibers to the prefrontal cortex. The thalamo-cortico-striatal projection system is involved in higher-order cognitive processes, including calculating the required effort and reward for engagement in a behavioral task. [55] From a behavioral perspective, fatigue is often described as a difficulty in initiating and sustaining voluntary activities.[4] Thus, regional damage to the anterior and/or dorsomedial nuclei and their frontal projections may dispose an individual to increased levels of fatigue post-TBI. Indeed, prior work from our group has shown greater level of post-mTBI fatigue was associated with reduced white matter integrity values in the anterior internal capsule—a fiber pathway with connections between the thalamus and the frontal lobe. [56] Moreover, in studies of aging, lower thalamic volume has been shown to be significantly associated with decreased thalamic white matter microstructure,[57,58] especially in regions with fronto-cortico projections.[59]

Our regional thalamic morphometry results dovetail with findings of thalamic atrophy and increased rates of neuronal loss observed in moderate to severe TBI samples [26,29,60] and may be suggestive of a regional vulnerability to injury. While the precise mechanisms underlying thalamic injury are unclear, primary axonal injury may initiate secondary neuroinflammatory processes that contribute to thalamic damage post-injury. [16,26] Indeed, when compared to controls with no history of head trauma, persistent microglial activation—a direct marker of inflammation—was observed in the thalamus of individuals with history of moderate to severe

TBI. [27] Importantly, microglial activation within the thalamus correlated with degree of thalamo-cortical tract damage. Given the anterior and dorsomedial body of the thalamus have dense connections with cortical association and projection fibers, these regions may therefore be vulnerable sites of Wallerian and retrograde degeneration post-injury. [27]

Although we chose to focus on the role of both global and regional thalamic morphometry in subjective fatigue post-mTBI, it is important to acknowledge that the underlying network of fatigue is broad and other basal ganglia and limbic structures have been implicated. [4,17,50,51] Therefore, we conducted a series of post-hoc analyses to explore potential associations between fatigue and global volumetric and regional morphometry of the caudate, putamen, nucleus accumbens, and globus pallidus using FSL's FIRST output. Parallel statistical models controlling for age, ICV, sex, PCL-M total score, and the BDI Affective subscale total revealed no significant associations between fatigue and lateralized volumes of the caudate, putamen, nucleus accumbens, and globus pallidus (p's ranged from .12 - .57. No significant associations between fatigue and regional morphometry of lateralized putamen, nucleus accumbens, globus pallidus, and the right caudate (all p's >.05) were observed. However, higher levels of fatigue were significantly associated with localized reductions in the head and medial surface of left caudate (p <. 05). The caudate is part of the ventral striatum, receiving projections from the frontal lobe, and also plays a contributory role in effort-reward valuations. Importantly, studies in TBI and other clinical populations have revealed that structural[18,19,52,61] and functional alterations[62-65] to the caudate have been associated with fatigue. While the mechanisms underlying caudate dysfunction are unclear, it, too, may be susceptible to direct trauma or the same negative neuroinflammatory processes. Alternatively, damage to the frontal or basal ganglia dopaminergic system may negatively alter caudate response.[66]

Increased rates of psychiatric symptoms are also common post-TBI [2,8,67] and, while distinct, there is considerable overlap between the clinical symptoms of depression, PTSD, and fatigue. [8,9,68] Additionally, structural and functional alterations to fronto-limbic and fronto-striatal structures have been linked to increased psychiatric symptom severity post-TBI.[69,70] Our results showed that, independent of PTSD and depressive symptoms, fatigue was associated with both global and regional thalamic morphometry. Importantly, neither PTSD nor depressive symptoms were significant predictors of thalamic morphometry in our regression models. Moreover, parallel analyses conducted in the putamen, nucleus accumbens, globus pallidus, and caudate revealed no significant associations between volume, morphometry, and psychiatric symptoms. These results therefore suggest that neurostructural alterations to the thalamus (and caudate) are specifically linked to fatigue post-TBI and not psychiatric symptoms in general. Importantly, these findings help assist in clarifying the nature and underlying cause of specific post-concussive symptoms in those with history of mild TBI.

Within the mTBI literature, there is some evidence to suggest that negative brain or behavior consequences differ as a function of type of injury or number of mTBIs, time since injury, or cumulative exposure to blast.[71-74] Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime, with the vast majority of these additional injuries involving an AOC versus an LOC. Moreover, there was considerable variability across the sample in time since their most recent mTBIs and whether they were exposed to blast while on deployment. Teasing apart differential associations between single vs. multiple TBIs within the context of this study is challenging given that only a small proportion of the sample that experienced a single TBI (i.e., only 22%) during their lifetime. However, we conducted a series of secondary analyses in which total number of TBIs, time since most recent TBI, and total

number of blast exposures within 100 meters were included as separate covariates in our regression analyses; results revealed that none of these variables were significantly associated with our dependent variables, and all significant fatigue, thalamic volume and morphometry findings held.

To our knowledge, the current study represents the first to directly examine the relationship between fatigue and thalamic morphometry in a sample of Veterans with history of mTBI. Strengths of this study include a relatively large sample of well-characterized Veterans with head trauma histories and investigation of both global and regional thalamic morphometry. However, our study has some weaknesses that should be noted. First, other regions may also be important to investigate in future studies—including projections to and between structures involved in those related to the broader circuit loops described above. Second, as is commonly a limitation in TBI research, our diagnosis of mTBI was based on retrospective self-report and may therefore be subject to recall bias. That said, this bias is mitigated by our comprehensive TBI assessment that takes into account head injuries an individual may have prior to, during, and after their military service. Moreover, to ensure comprehensive and reproducible characterizations, we applied strict VA/DoD diagnostic guidelines[30] to all reported injuries, which will augur for generalizability to other studies that employ the same guidelines. Finally, we collapsed across total number and mechanisms of injury when conducting our analyses; however, future studies are needed in order to tease apart whether or how thalamic morphometry may differ between those with blunt versus blast-related TBI only.

CONCLUSION

In conclusion, fatigue is a common and oftentimes chronic and disabling symptom following TBI. Results from our study align with findings from other clinical populations to

show that the thalamus is a critical structure involved in the manifestation of fatigue. In particular, we found that lower thalamic volumes and reduced regional morphometry in areas containing higher-order nuclei (i.e., antero-dorso-medial aspects of the thalamic body) resulted in greater levels of fatigue in Veterans with mTBI. Future studies should investigate components of fatigue (e.g., physical and cognitive) and integrate multiple MR methods in order to clarify and expand our understanding of thalamic damage and concomitant behavioral consequences in the aftermath of TBI.

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Table. 1 Sample Characteristics

	Mean (SD)
Age	31.86 (6.43)
Education	14.19 (1.60)
WRAT-4 Reading Standard Score	101.48 (10.92)
Sex (% Male)	87%
Ethnicity Caucasian African American Hispanic Asian	 44% 8% 35% 13%
% Of individuals with history of one versus multiple TBIs	22% vs. 78%
Total Number of Lifetime TBIs	2.57 (1.45), *Median* = 2
Time Since Most Recent TBI (months)	63.67 (42.64), *Median* = 58
Time Since Most Significant TBI (months)	70.98 (43.94), *Median* = 67
% With exposed to blast within 100 meters	60%
% With LOC for most Significant Injury	62%
Most Significant TBI Type % Blast % Blunt % Blast with secondary/tertiary Blunt	 19% 67% 14%
Combat Exposure Scale Total	15.75 (11.78)
Modified Fatigue Impact Scale Total	41.16 (19.82)
Post-Traumatic Stress Disorder Checklist Total	43.68 (18.78)
Beck Depression Inventory-II Total	20.77 (12.81)
Beck Depression Inventory-II Affective Subscale Total	9.59 (7.14)
Neurobehavioral Symptom Inventory Total	33.11 (18.18)
Pittsburgh Sleep Quality Index Global Score (n = 57)	10.89 (4.43)

WRAT-4 = Wide Range Achievement Test-4th Edition; TBI = traumatic brain injury; NSI = Neurobehavioral Symptom Inventory; LOC = loss of consciousness

Table 2. Multiple Hierarchical Linear Regression Models for Right Thalamic Volume

	Variable	B	*SE*	β	*p*	F	R^2	Δ R^2
Right Thalamus						4.57	.29	
Block 1	Age	-39.32	13.50	-.333	.005			
	Sex	-529.23	265.80	-.234	.051			
	ICV	1139.69	404.65	.327	.007			
	BDI-II Affective Subscale Total	-10.24	17.52	-.096	.561			
	PCL-M Total	5.42	6.75	.134	.425			
Block 2	Age	-36.18	13.09	-.306	.008	5.32	.35	.062
	Sex	-532.50	256.27	-.235	.042			
	ICV	825.19	413.28	.237	.051			
	BDI-II Affective Subscale Total	2.93	17.83	.027	.870			
	PCL-M Total	12.17	7.13	.301	.093			
	MFIS Total Score	-14.83	6.43	-.387	.025			

Table 2. Multiple Hierarchical Linear Regression Models for Left Thalamic Volume

	Variable	B	*SE*	β	*p*	F	R^2	Δ R^2
Left Thalamus						4.95	.30	
Block 1	Age	-38.87	12.80	-.343	.004			
	Sex	-625.79	252.11	-.288	.016			
	ICV	991.75	383.83	.297	.012			
	BDI-II Affective Subscale Total	-1.98	16.62	-.019	.905			
	PCL-M Total	.620	6.40	.016	.923			
Block 2	Age	-36.21	12.54	-.320	.005	5.04	.35	.048
	Sex	-628.56	245.43	-.290	.013			
	ICV	725.72	395.81	.217	.072			
	BDI-II Affective Subscale Total	9.16	17.08	.090	.594			
	PCL-M Total	6.33	6.83	.163	.358			
	MFIS Total Score	-12.54	6.16	-.341	.046			

Figure 1. Partial Regression Plot for Association Between Fatigue and Right Thalamic Volume

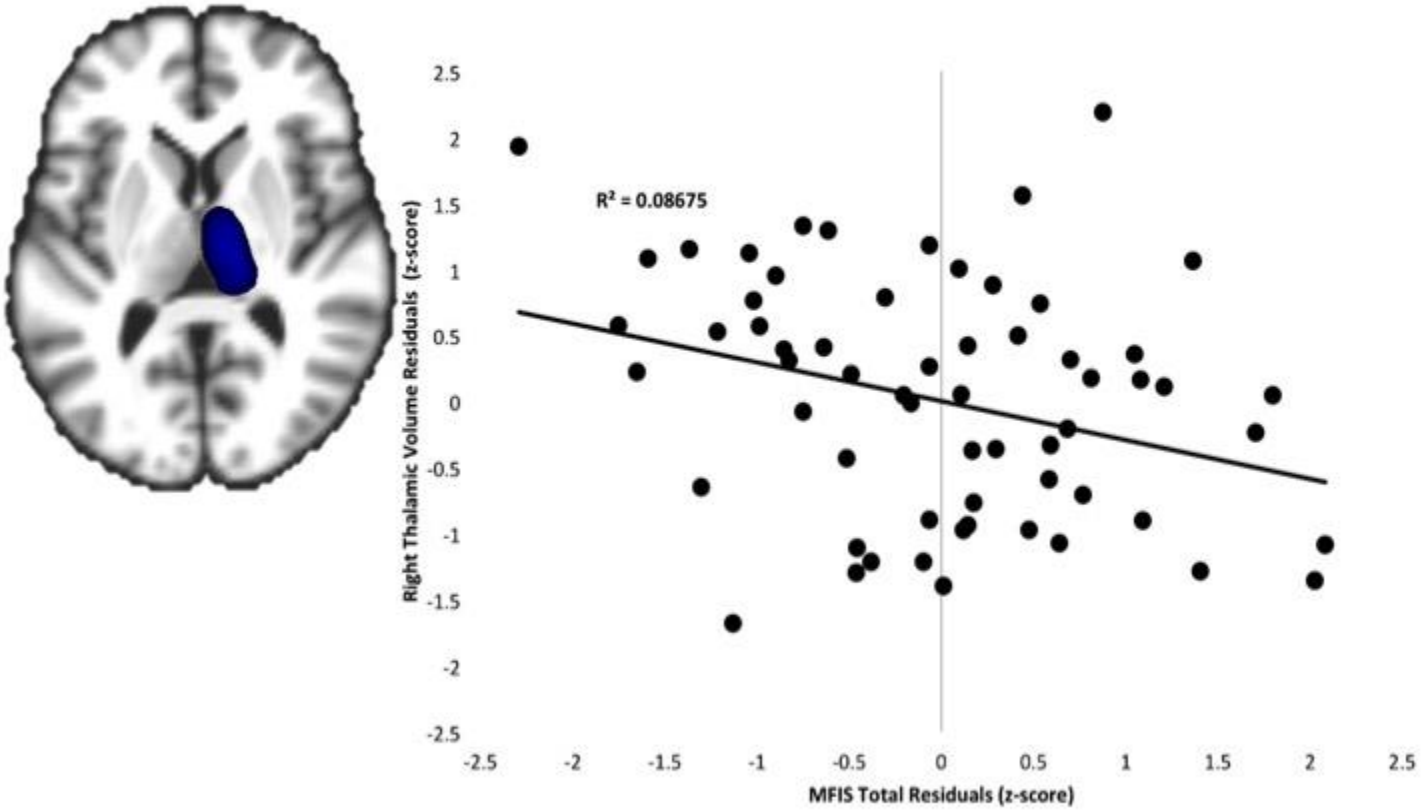


Figure 2. Partial Regression Plot for Association between Fatigue and Left Thalamic Volume

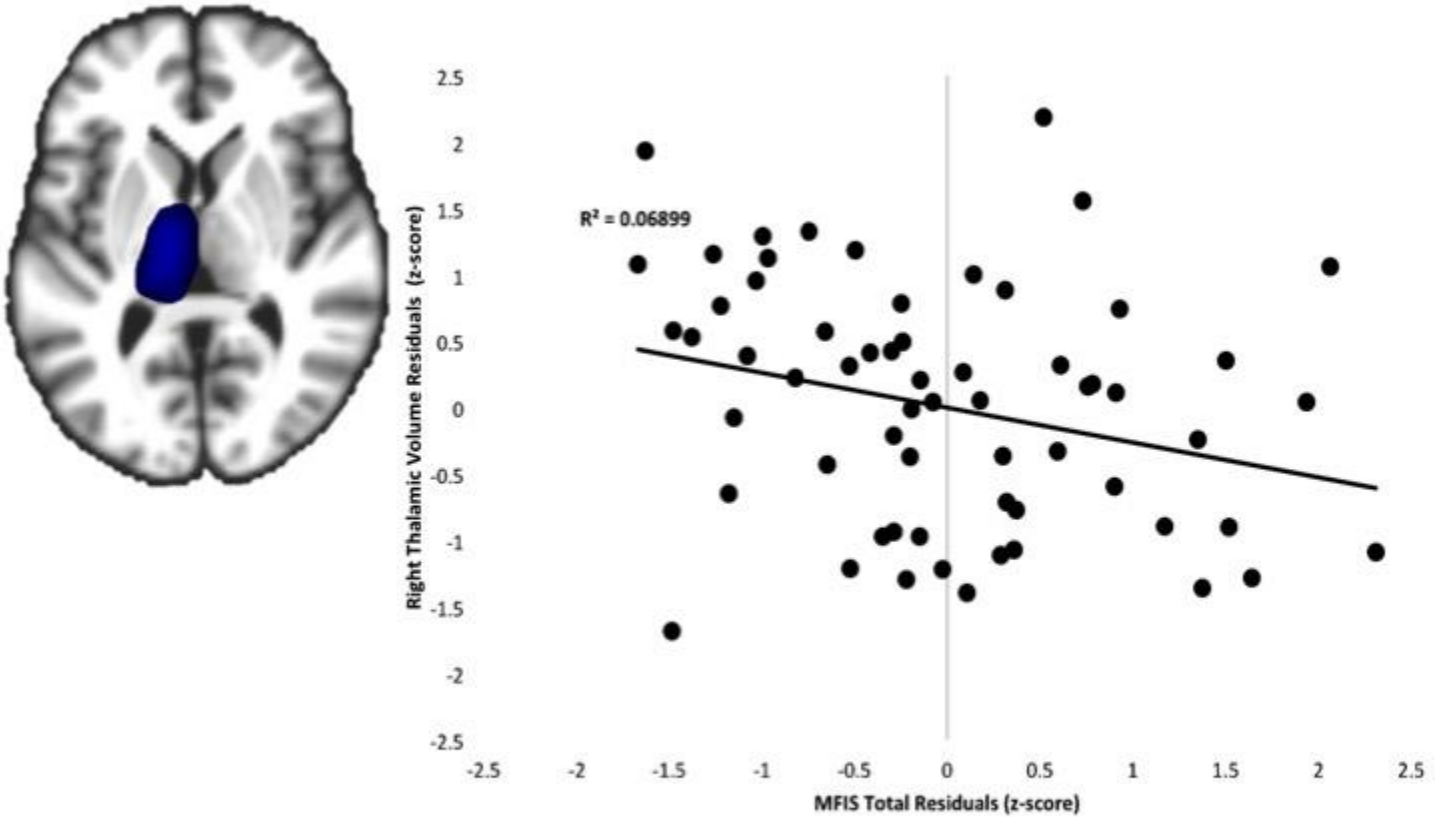
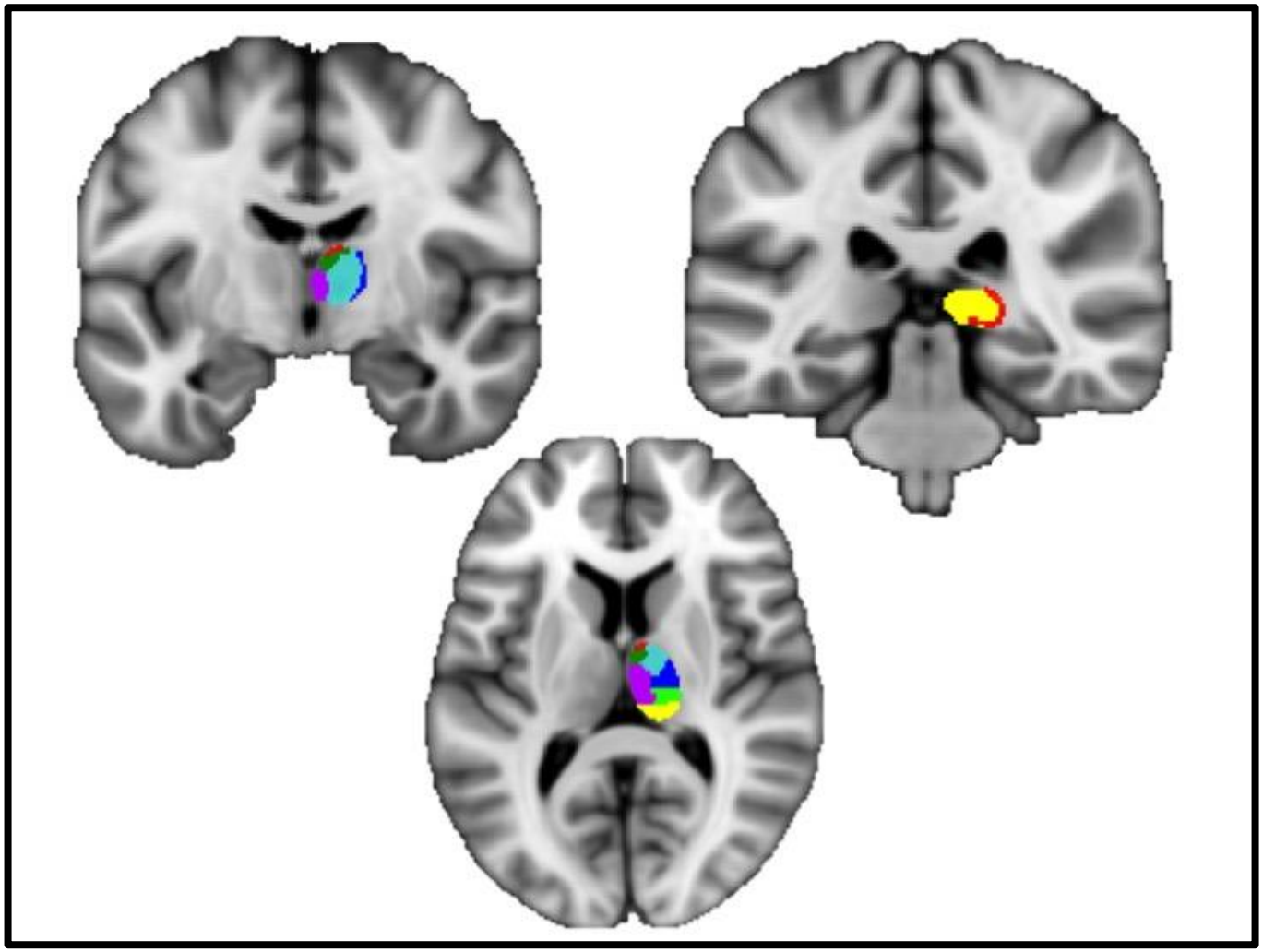
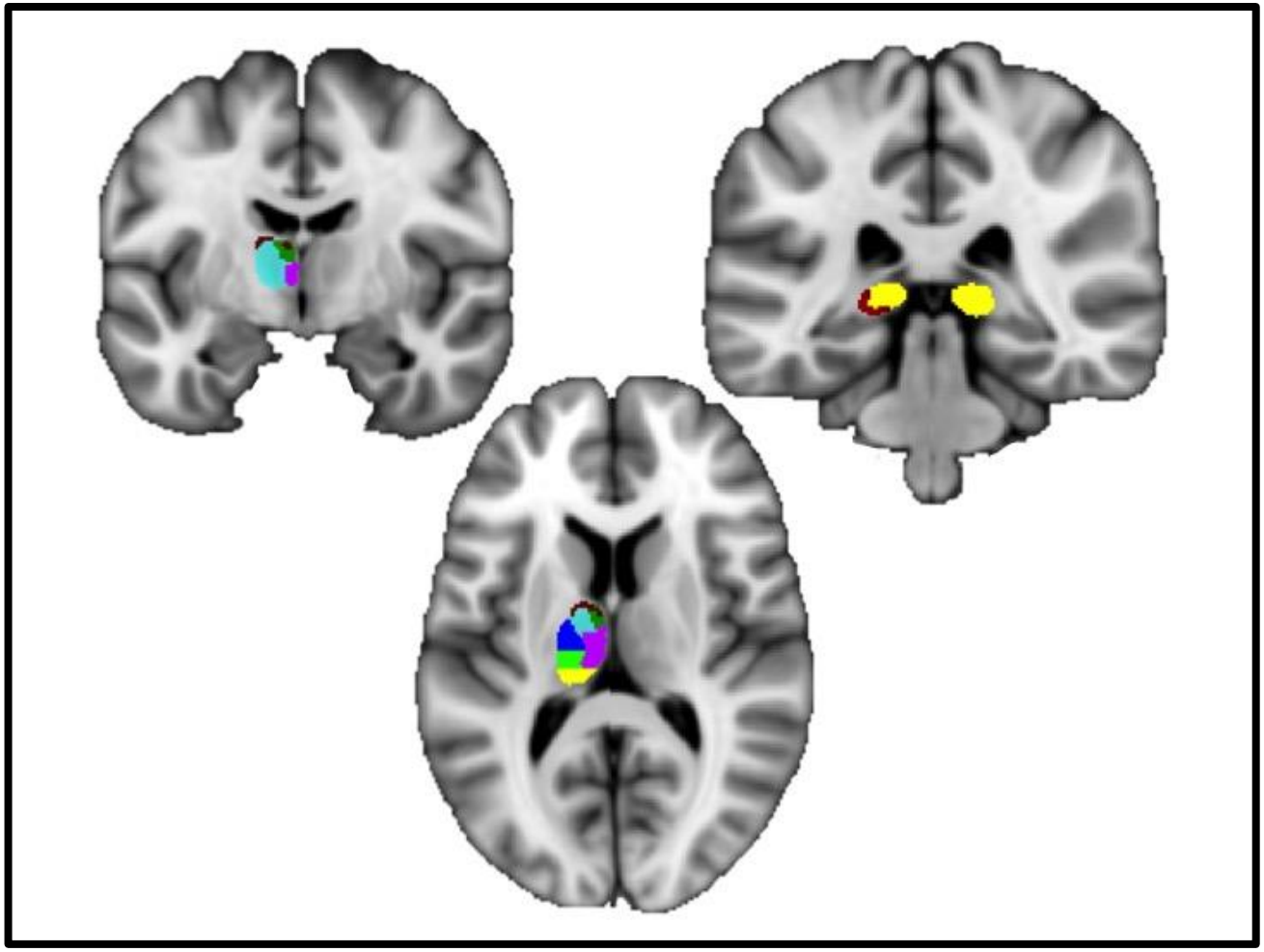


Figure 3. Regional Atrophy for Right Thalamus



Significant areas of atrophy are depicted in red ($p < .05$). Other colored areas are ICBM T1 Atlas parcellations of thalamus. Dark Green = Anterior Nucleus, Light Green = Lateral Posterior Nucleus, Dark Blue = Ventral Lateral Nucleus, Light Blue = Reticular Nucleus, Purple = Ventral Anterior Nucleus, Yellow = Dorsomedial Nucleus

Figure 4. Regional Atrophy for Left Thalamus



Significant areas of atrophy are depicted in dark red ($p < .01$). Other colored areas are ICBM T1 Atlas parcellations of thalamus. Dark Green = Anterior Nucleus, Light Green = Lateral Posterior Nucleus, Dark Blue = Ventral Lateral Nucleus, Light Blue = Reticular Nucleus, Purple = Ventral Anterior Nucleus, Yellow = Dorsomedial Nucleus

Fatigue is Associated with Global and Regional Thalamic Morphometry in Veterans with History of Mild Traumatic Brain Injury

Word Count: 4,254
Key Words: fatigue, central fatigue, thalamus, thalamic morphometry, thalamic volume, thalamic shape, mild traumatic brain injury, mTBI, Veterans

ABSTRACT

Objective: Fatigue is a complex, multidimensional phenomenon that commonly occurs following traumatic brain injury (TBI). The thalamus—a structure vulnerable to both primary and secondary injury in TBI—is thought to play a pivotal role in the manifestation of fatigue. We explored how neuroimaging markers of local and global thalamic morphometry relate to the subjective experience of fatigue post-TBI. **Methods:** 63 Veterans with history of mild TBI (mTBI) underwent structural magnetic resonance scanning (MRI) and completed questionnaires related to fatigue and psychiatric symptomatology. FMRIB's Software (FSL) was utilized to obtain whole brain and thalamic volume estimates, as well as to perform regional thalamic morphometry analyses. **Results:** Independent of age, sex, intracranial volume, PTSD and depressive symptoms, greater levels of self-reported fatigue was significantly associated with decreased right ($p = .026$) and left ($p = .046$) thalamic volumes. Regional morphometry analyses revealed that fatigue was significantly associated with reductions in the anterior and dorsomedial aspects of the right thalamic body ($p < .05$). Similar trends were observed for the left thalamic body ($p < .10$). **Conclusions:** Both global and regional thalamic morphometric changes are associated with the subjective experience of fatigue in Veterans with history of mTBI. These findings support a theory in which disruption of thalamo-cortico-striatal circuitry may result in the manifestation of fatigue in individuals with history of neurotrauma.

INTRODUCTION

Central fatigue is a complex, multi-dimensional phenomenon that is common and frequently intractable in the aftermath of traumatic brain injury (TBI). [1-4] It is distinct from more *peripheral* categorizations of fatigue (i.e., disorders of the neuromuscular junction or muscular fatigue) given that it manifests as a result of *central* nervous system damage and/or dysfunction and it has been conceptualized as "the failure to initiate and/or sustain attentional tasks and physical activities requiring self-motivation."[5(p35)] Central fatigue (referred to as *fatigue* here within) has components that are both cognitive and physical [5] and, although it overlaps with psychiatric and sleep disturbances, fatigue has been demonstrated to be a distinct or independent construct. [6-8] Importantly, across all severities of TBI (i.e. mild, moderate, or severe), fatigue has been linked to worse outcomes, including increased levels of disability and poorer quality of life. [9-11]

Although the underlying neural network of fatigue remains to be fully characterized, there is a growing body of literature to suggest that thalamus may play a critical role. The thalamus—a subcortical gray matter structure that contains many groups of nuclei—is a critical relay station for a diffuse network of afferent and efferent projections within the brain. [12-14] It processes both motor and sensory information and contributes to high-order cognitive processes including memory and executive functions. [15,16] The thalamus is part of the ascending reticular activating system (ARAS), which ensures adequate levels of arousal needed to carry out various behavioral tasks [17] and is thus positioned to play a critical role in the manifestation of fatigue. In support of this position, structural changes to the thalamus have been linked to greater levels of fatigue across various clinical populations including multiple sclerosis and chronic fatigue syndrome. [18-20]

Within the context of TBI, the thalamus is susceptible to both primary and secondary mechanisms of injury.[21,22] Simulations from finite element head modeling have revealed the thalamus is a region of high shear stress during neurotrauma. [23,24] Furthermore, secondary neuroinflammatory and degenerative processes—which have been demonstrated to persist for many years following neurotrauma—also contribute to thalamic damage following TBI. [16,25-27] Structural magnetic resonance imaging (MRI) methods have been used to examine both global and more nuanced topographic, or regional, alterations of the thalamus post-TBI. Across samples of moderate to severe TBI, global reductions in thalamic volume and regional atrophy in antero-dorsal-medial aspects of the thalamus have been observed post-injury.[28] Moreover, regional changes in thalamic morphometry has been linked to greater rates of disability [29] and poorer performance on measures of executive control [21,26] in those with history of moderate to severe TBI.

Studies examining both global and regional morphometry in less severe TBI samples (i.e., mild TBI [mTBI]) are relatively limited. Global reductions in thalamic volume [30,31] have been observed and only one study has explored regional morphometry of the thalamus within an mTBI sample. [32] In contrast to the previous studies in moderate to severe TBI samples, regional reductions were limited to the posterolateral portion of the thalamus of the mTBI group relative to orthopedic-injured controls. [32] However, it remains unclear to what degree these alterations in thalamic morphometry may contribute to the subjective experience of fatigue post-mTBI. Therefore, we explored the potential role thalamic integrity may play in the manifestation of fatigue in mTBI. We hypothesized that greater levels of fatigue would be associated with both decreased volume as well as local atrophy in the thalamus in Veterans with history of mTBI.

METHODS

Participants were 63 Operation Enduring Freedom, Operation Iraqi Freedom, and Operation New Dawn (OEF/OIF/OND) Veterans with history of mTBI recruited from posted study advertisements and outpatient clinics at the XXX in XXX. The institutional review boards (IRBs) at the XX and XXX approved all study procedures. Prior to enrollment in the study, participants provided written and informed consent. TBI history interviews, neuropsychological testing, and study questionnaires were completed at the XXX located on the XXX campus. Brain MRI scans occurred at the XXX Center for Functional MRI.

The following exclusionary criteria were applied to the study sample overall: (1) moderate (loss of consciousness [LOC] >30 minutes but < 24 hours, and alteration of consciousness [AOC] > 24 hours or post-traumatic amnesia [PTA] >1 day but < 7 days) or severe (LOC ≥ 24 hours, AOC > 24 hours, or PTA ≥ 7 days) TBI per [33] guidelines; (2) current (within 30 days) alcohol or other substance abuse, (3) current or prior alcohol or substance dependence; (4) current or prior diagnosis of bipolar disorder, schizophrenia, or psychotic disorder as determined by the *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., DSM-IV, American Psychiatric Association, 2000); (5) a positive toxicology screen as measured by the Rapid Response 10-drug Test Panel; (6) prior history of a learning disability; (7) history of any other major neurological (e.g., multiple sclerosis, stroke, seizures) or medical conditions (e.g., chronic fatigue syndrome, diabetes, myocardial infarction) that may affect brain structure or cognition; (8) current suicidal and/or homicidal ideation; (9) involvement in current or pending litigation; (10) any contraindications to MRI scanning (e.g., presence of metal in one's body, or possible pregnancy for females participants); and (11) any gross abnormalities on structural MRI scans.

TBI History Interview and Diagnosis

Each participant underwent a TBI history interview that was conducted by a post-baccalaureate or graduate-level research assistant. TBI history interviews included assessment of both non-military (i.e., prior to or after discharge from the military) and military-related (i.e., during enlistment in the US armed services) head injuries (see [34] for more comprehensive details). This interview was adapted from the VA Structured Clinical Interview for TBI[35] and involves assessment of any injuries in which participants may have fallen, suffered a blow to the head, or experienced a blast-wave detonation (i.e., overpressure or shock waves resulting from an explosive detonation) within 100 meters (i.e., length of a professional football field). In brief, for each reported injury, participants were queried about: presence and duration of critical injury severity charactertistics (i.e., LOC, AOC, and PTA), the nature of the accident (e.g, fight or motor vechicle accient), the date the injury occurred, the mechanism of injury (i.e., blunt/mechanical or blast-related forces), and the presence and duration of any post-concussive symptoms. Diagnosis of mTBI was based on the VA/DoD guidelines [33] of a loss of consciousness (LOC) < 30 minutes, or alteration of consciousness (AOC) or posttraumatic amnesia (PTA) < 24 hours. The sum of all injuries that met VA/DoD diagnostic criteria for mTBI was calculated to determine the total number of lifetime TBIs each participant experienced. Additionally, injury severity characteristics (i.e., LOC, AOC, PTA) were also utilized to determine the "most significant" or "worst" TBI; this involved direct comparisons of LOC and AOC durations for each injury, and injuries where a LOC was sustained were considered more significant/severe than those that had an AOC only. Finally, the time between participants' most recent and significant TBI and their neuropsychological testing date was calculated."

Assessment of Fatigue

The Modified Fatigue Impact Scale (MFIS) was used to assess current levels of fatigue and its impact on functioning over the past four weeks. The MFIS is a 21-item scale modified from the original 40-item Fatigue Impact Scale [36] that was recently validated for use in Veterans with history of mild or moderate TBI. [37] Items from the MFIS can be summed to generate a total score (ranging from 0 to 84), with higher scores indicating greater effects of fatigue on both cognitive and physical functions.

Psychiatric Symptom Rating Scales and Other Self-Report Questionnaires

Participants completed symptom rating scales that quantified current levels of posttraumatic stress (PTSD Checklist [PCL-M] [38]), depression (Beck-Depression Inventory-II [BDI-II] [39]), and post-concussive symptoms (Neurobehavioral Symptom Inventory [NSI] [40]). For the BDI-II, an affective subscale [1,41] was also generated for use in subsequent analyses given some items overlap with the characterization of fatigue. Exposure to various combat situations and wartime stressors during deployment was assessed using the Combat Exposure Scale ([CES] [42]). Overall sleep quality for the past month was assessed with the Pittsburgh Sleep Quality Index ([PSQI] [43]).

Neuroimaging Data Acquisition

Structural MRI scans were acquired on a 3-Tesla General Electric MR750 system with an eight-channel head coil. A high-resolution T1 anatomical scan was acquired in the sagittal plane with the following parameters: FOV = 24 cm, 256 x 192 matrix, TR = 8.1 ms, TE = 3.192 ms, flip angle = 12°, TI = 550 ms, bandwidth = 31.25 kHz, and 172 1.2 mm slices. After the images were acquired, they were visually inspected for quality control purposes to ensure there were no artifacts that might affect image processing (e.g., motion).

Neuroimaging Processing & Analyses

Images were processed and analyzed using FMRIB Software Library version 5.0. [44,45] Thalamic segmentation and shape analysis was performed with FMRIB Software Library's Integrated Registration and Segmentation Tool ([FIRST]; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST) which has been demonstrated to have the highest concordance among subcortical structures when compared to manual segmentation methods. [46] FIRST uses a two-stage affine process to register each participant's T1 onto an MNI152 template. In the first step, a whole brain registration was performed. Next, a weighted subcortical mask was applied to the intermediate image, which then underwent another affine registration to ensure optimal registration of subcortical structures. The inverse transformation of the estimated registration was then calculated and applied to the MNI152 template to bring it into native space. A Bayesian Active Appearance Model (AAM), which takes into account both geometric shape (i.e., morphometry) and image intensity, was used to register and segment subcortical structures.[43] After registration, a parameterized surface mesh for each subcortical structure is generated. FSL's *–useReconNative* and *-useRigidAlign* options were used to recreate the mesh in native space and remove pose effects. Registrations and thalamic segmentations were checked with FSL's *slicesdir* command.

Global and Regional Thalamic Morphometry

Thalamic volume estimates were extracted from the FIRST segmentation. Intracranial volume (ICV) estimation occurred through the FMRIB Software Library [44,45] using ENGIMA imaging protocol guidelines (http://enigma.ini.usc.edu). FIRST also enables examination of localized morphometric differences in subcortical structures. A deformable surface mesh model composed of sets of vertices is generated for each subject and allows for the mean shape and any

deviations from the mean to be explored. As such, regional morphometry analyses examine any expansions and reductions from the TBI group's mean surface level of the thalamic body. Reduced regional morphometry is associated with negative perpendicular distances from the average surface and expansions represent positive perpendicular distances. [46] In our study, we conducted vertex-wise analyses using general linear modeling (GLM) to explore thalamic morphometry and fatigue associations in the sample. The International Consortium for Brain Mapping Atlas (ICBM) T1 Atlas, [47] which parcellates the thalamus into 11 structurally defined regions of interest (ROIs), was then used to provide additional context about thalamic subnuclei likely involved in the observed regional findings. Prior to overlaying the regional thalamic findings to determine the overlapping ROIs, the ICBM Atlas was registered to MNI-152 space using FSL's FMRIB's Linear Image Registration Tool (FLIRT). ICV was calculated by multiplying the size of the template brain by the inverse of the determinant of the affine matrix.

Statistical Analyses

Hierarchical linear regressions were used to determine whether fatigue was predictive of thalamic volumes above and beyond age, sex, ICV, PCL-M and BDI-II Affective subscale scores using the Statistical Package for the Social Sciences (SPSS) version 21. [48] We entered age, ICV, and sex into Block 1 of our regression models given their known association with brain volume estimates. The PCL-M and BDI-II Affective subscale were also entered into Block 1 since we were interested in the effects of fatigue—independent of other common psychiatric comorbidities that have been shown across several studies to have some symptom overlap—on thalamic volumes. Multicollinearity of the independent variables was assessed and all variance inflation factor (VIF) values were less than 3. FMIRB's GLM tool (www.fmrib.ox.ac.uk/fsl/fsl/list) was used to explore associations between fatigue and thalamic

morphometry while controlling for age, ICV, PCL-M and BDI-II Affective subscale scores. For regional thalamic morphometry analyses, FSL's Randomise tool (www.fmrib.ox.ac.uk/fsl/randomise) was used to test correlation inferences with permutation methods. [49,50] Positive and negative associations for thalamic morphometry and fatigue were explored with 5,000 two-tailed Monte Carlo permutation tests with threshold-cluster free enhancement to correct for multiple comparisons at $p = .05$.

RESULTS

Sample characteristics are presented in Table 1. On average, participants were relatively young (mean age = 31.9), predominantly male (87%), experienced multiple mTBIs (78%), and were many months removed from their most recent injury (mean time = 63.7 months). With respect to their most significant TBI, 58% of these injuries occurred during deployment, 62% involved a LOC versus an AOC, and 67% were due primarily to blunt-force trauma. Of the individuals with a history of multiple TBIs, approximately 18% reported another injury that involved an LOC versus 82% involved an AOC.

[INSERT TABLE 1 AND 2 AROUND HERE]

Association Between Fatigue and Thalamic Volume

Hierarchical regressions were performed in an effort to determine whether fatigue was predictive of thalamic volume for each hemisphere. Age, ICV, sex, PCL-M total score, and the BDI-II Affective subscale total were entered into Block 1, and the MFIS total score was entered into Block 2 of the models. Results are presented in Table 2. Block 1 explained 28.6% of the variance in right thalamic volume. A significant increase in the amount of variance in right thalamic volume was observed when the MFIS total score was added into Block 2 of the model ($R^2 \Delta = .062$, $F \Delta (1,56) = 5.32$, $p = .025$). Results indicated that lower right thalamic volumes

were associated with higher levels of fatigue (see Figure 1). For left thalamic volume, Block 1 explained 30.3% of the variance, which was also increased with the addition of MFIS total score into Block 2 of the model (R^2 Δ = .048, F Δ (1,56) = 4.15, p = .046). Results indicated that lower left thalamic volumes were associated with higher levels of fatigue. See Figure 2.

[INSERT FIGURE 1 AROUND HERE]

Associations Between Fatigue and Regional Thalamic Morphology

To test for an association between fatigue and regional thalamic morphometry, a series of regression analyses were performed with FSL's GLM and *randomise* tool. Significant negative correlations between fatigue and right thalamic morphometry were found, even after adjusting for age, ICV, sex, PCL-M total score, and the BDI-II Affective subscale total. See Figure 3 for the significant clusters. Results were overlaid onto the ICBM Atlas to provide additional context about the thalamic nuclei that correspond to the atrophied areas. In particular, reductions in the anterior and dorso-medial aspects of the right thalamus were associated with higher levels of self-reported fatigue (p's < .05). With respect to the left thalamus, there were no significant associations between morphometry and fatigue that survived multiple comparisons (p's > .05). However, when alpha was adjusted to p < .10 to explore potential trends, associations between higher levels of fatigue and localized reductions in the anterior, dorsomedial, and reticular aspects of the left thalamus were observed. See Figure 4.

[INSERT FIGURE 2 AROUND HERE]

DISCUSSION

Our study provides evidence that the thalamus—a subcortical gray matter nucleus responsible for processing much of our sensory, motor, cognitive and emotional information, and coordinating behavior[51]—is involved in the subjective experience of fatigue following mTBI. In

particular, we found that greater levels of fatigue were associated with (1) decreased thalamic volumes and (2) regional reductions in the anterior and dorsomedial aspects of the thalamic body. Importantly, these findings implicate the thalamus as an important structure involved in the underlying neural network of fatigue. Results dovetail with findings from other clinical populations (i.e., multiple sclerosis) demonstrating that the thalamus is involved in the manifestation of fatigue-related symptoms.[18,19,52] Moreover, given careful consideration of important covariates and possible confounds, our results suggest that neurostructural alterations to the thalamus are specifically linked to fatigue post-TBI and not psychiatric symptoms in general.

Chaudhuri and Behan [5] described a model in which disruption and/or dysfunction of the neural circuitry of the thalamo-cortico-striatal loop is thought to result in fatigue. They argue that, when this circuit is functioning normally, there is a positive feedback loop in which: (1) glutamatergic (excitatory) signals from the frontal cortex lead to excitation of the striatum, (2) striatal activation increases the GABAergic (inhibitory) signals of the substantia nigra and globus pallidus, which then (3) decreases the inhibitory signals of the globus pallidus and substantia nigra on the thalamus, resulting in (4) excitatory output from the thalamus to the frontal cortex (see [53] for comprehensive review of this pathway). *Global* thalamic volume loss may thus cause a net change in thalamic activity, and therefore disrupt the behavioral functions associated with these said pathways. Indeed, relative to controls with no history of head-trauma, resting-state functional MRI has revealed decreased thalamic activity [31] and abnormal thalamo-cortical connectivity patterns in mTBI patients. [14,54]

Results from our study revealed that greater levels of fatigue were associated with reduced *regional* thalamic morphometry in the anterior and dorsomedial body of the thalamus.

Regarding neuroanatomical connections, the anterior nucleus projects efferent fibers to the oribitofrontal and cingulate cortices, while the dorsomedial nucleus sends fibers to the prefrontal cortex. The thalamo-cortico-striatal projection system is involved in higher-order cognitive processes, including calculating the required effort and reward for engagement in a behavioral task. [55] From a behavioral perspective, fatigue is often described as a difficulty in initiating and sustaining voluntary activities.[4] Thus, regional damage to the anterior and/or dorsomedial nuclei and their frontal projections may dispose an individual to increased levels of fatigue post-TBI. Indeed, prior work from our group has shown greater level of post-mTBI fatigue was associated with reduced white matter integrity values in the anterior internal capsule—a fiber pathway with connections between the thalamus and the frontal lobe. [56] Moreover, in studies of aging, lower thalamic volume has been shown to be significantly associated with decreased thalamic white matter microstructure,[57,58] especially in regions with fronto-cortico projections.[59]

Our regional thalamic morphometry results dovetail with findings of thalamic atrophy and increased rates of neuronal loss observed in moderate to severe TBI samples [26,29,60] and may be suggestive of a regional vulnerability to injury. While the precise mechanisms underlying thalamic injury are unclear, primary axonal injury may initiate secondary neuroinflammatory processes that contribute to thalamic damage post-injury. [16,26] Indeed, when compared to controls with no history of head trauma, persistent microglial activation—a direct marker of inflammation—was observed in the thalamus of individuals with history of moderate to severe TBI. [27] Importantly, microglial activation within the thalamus correlated with degree of thalamo-cortical tract damage. Given the anterior and dorsomedial body of the thalamus have dense connections with cortical association and projection fibers, these regions may therefore be vulnerable sites of Wallerian and retrograde degeneration post-injury. [27]

Although we chose to focus on the role of both global and regional thalamic morphometry in subjective fatigue post-mTBI, it is important to acknowledge that the underlying network of fatigue is broad and other basal ganglia and limbic structures have been implicated. [4,17,50,51] Therefore, we conducted a series of post-hoc analyses to explore potential associations between fatigue and global volumetric and regional morphometry of the caudate, putamen, nucleus accumbens, and globus pallidus using FSL's FIRST output. Parallel statistical models controlling for age, ICV, sex, PCL-M total score, and the BDI Affective subscale total revealed no significant associations between fatigue and lateralized volumes of the caudate, putamen, nucleus accumbens, and globus pallidus (*p*'s ranged from .12 - .57. No significant associations between fatigue and regional morphometry of lateralized putamen, nucleus accumbens, globus pallidus, and the right caudate (all *p*'s >.05) were observed. However, higher levels of fatigue were significantly associated with localized reductions in the head and medial surface of left caudate ($p < .05$). The caudate is part of the ventral striatum, receiving projections from the frontal lobe, and also plays a contributory role in effort-reward valuations. Importantly, studies in TBI and other clinical populations have revealed that structural[18,19,52,61] and functional alterations[62-65] to the caudate have been associated with fatigue. While the mechanisms underlying caudate dysfunction are unclear, it, too, may be susceptible to direct trauma or the same negative neuroinflammatory processes. Alternatively, damage to the frontal or basal ganglia dopaminergic system may negatively alter caudate response.[66]

Increased rates of psychiatric symptoms are also common post-TBI [2,8,67] and, while distinct, there is considerable overlap between the clinical symptoms of depression, PTSD, and fatigue. [8,9,68] Additionally, structural and functional alterations to fronto-limbic and fronto-striatal structures have been linked to increased psychiatric symptom severity post-TBI.[69,70] Our results

showed that, independent of PTSD and depressive symptoms, fatigue was associated with both global and regional thalamic morphometry. Importantly, neither PTSD nor depressive symptoms were significant predictors of thalamic morphometry in our regression models. Moreover, parallel analyses conducted in the putamen, nucleus accumbens, globus pallidus, and caudate revealed no significant associations between volume, morphometry, and psychiatric symptoms. These results therefore suggest that neurostructural alterations to the thalamus (and caudate) are specifically linked to fatigue post-TBI and not psychiatric symptoms in general. Importantly, these findings help assist in clarifying the nature and underlying cause of specific post-concussive symptoms in those with history of mild TBI.

Within the mTBI literature, there is some evidence to suggest that negative brain or behavior consequences differ as a function of type of injury or number of mTBIs, time since injury, or cumulative exposure to blast.[71-74] Notably, 78% of this sample reported experiencing more than one mTBI during their lifetime, with the vast majority of these additional injuries involving an AOC versus an LOC. Moreover, there was considerable variability across the sample in time since their most recent mTBIs and whether they were exposed to blast while on deployment. Teasing apart differential associations between single vs. multiple TBIs within the context of this study is challenging given that only a small proportion of the sample that experienced a single TBI (i.e., only 22%) during their lifetime. However, we conducted a series of secondary analyses in which total number of TBIs, time since most recent TBI, and total number of blast exposures within 100 meters were included as separate covariates in our regression analyses; results revealed that none of these variables were significantly associated with our dependent variables, and all significant fatigue, thalamic volume and morphometry findings held.

To our knowledge, the current study represents the first to directly examine the relationship between fatigue and thalamic morphometry in a sample of Veterans with history of mTBI. Strengths of this study include a relatively large sample of well-characterized Veterans with head trauma histories and investigation of both global and regional thalamic morphometry. However, our study has some weaknesses that should be noted. First, other regions may also be important to investigate in future studies—including projections to and between structures involved in those related to the broader circuit loops described above. Second, as is commonly a limitation in TBI research, our diagnosis of mTBI was based on retrospective self-report and may therefore be subject to recall bias. That said, this bias is mitigated by our comprehensive TBI assessment that takes into account head injuries an individual may have prior to, during, and after their military service. Moreover, to ensure comprehensive and reproducible characterizations, we applied strict VA/DoD diagnostic guidelines[30] to all reported injuries, which will augur for generalizability to other studies that employ the same guidelines. Finally, we collapsed across total number and mechanisms of injury when conducting our analyses; however, future studies are needed in order to tease apart whether or how thalamic morphometry may differ between those with blunt versus blast-related TBI only.

CONCLUSION

In conclusion, fatigue is a common and oftentimes chronic and disabling symptom following TBI. Results from our study align with findings from other clinical populations to show that the thalamus is a critical structure involved in the manifestation of fatigue. In particular, we found that lower thalamic volumes and reduced regional morphometry in areas containing higher-order nuclei (i.e., antero-dorso-medial aspects of the thalamic body) resulted in greater levels of fatigue in Veterans with mTBI. Future studies should investigate components of

fatigue (e.g., physical and cognitive) and integrate multiple MR methods in order to clarify and expand our understanding of thalamic damage and concomitant behavioral consequences in the aftermath of TBI.

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	Mean (SD)
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Sex (% Male)	87%
Ethnicity Caucasian African American Hispanic Asian	 44% 8% 35% 13%
% Of individuals with history of one versus multiple TBIs	22% vs. 78%
Total Number of Lifetime TBIs	2.57 (1.45), *Median* = 2
Time Since Most Recent TBI (months)	63.67 (42.64), *Median* = 58
Time Since Most Significant TBI (months)	70.98 (43.94), *Median* = 67
% With exposed to blast within 100 meters	60%
% With LOC for most Significant Injury	62%
Most Significant TBI Type % Blast % Blunt % Blast with secondary/tertiary Blunt	 19% 67% 14%
Combat Exposure Scale Total	15.75 (11.78)
Modified Fatigue Impact Scale Total	41.16 (19.82)
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Beck Depression Inventory-II Total	20.77 (12.81)
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569 WRAT-4 = Wide Range Achievement Test-4th Edition; TBI = traumatic brain injury; NSI =
570 Neurobehavioral Symptom Inventory; LOC = loss of consciousness

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Table 2. Multiple Hierarchical Linear Regression Models for Right Thalamic Volume

	Variable	B	*SE*	β	*p*	F	R^2	Δ R^2
Right Thalamus						4.57	.29	
Block 1	Age	-39.32	13.50	-.333	.005			
	Sex	-529.23	265.80	-.234	.051			
	ICV	1139.69	404.65	.327	.007			
	BDI-II Affective Subscale Total	-10.24	17.52	-.096	.561			
	PCL-M Total	5.42	6.75	.134	.425			
Block 2	Age	-36.18	13.09	-.306	.008	5.32	.35	.062
	Sex	-532.50	256.27	-.235	.042			
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	MFIS Total Score	-14.83	6.43	-.387	.025			

Table 2. Multiple Hierarchical Linear Regression Models for Left Thalamic Volume

	Variable	B	*SE*	β	*p*	F	R^2	Δ R^2
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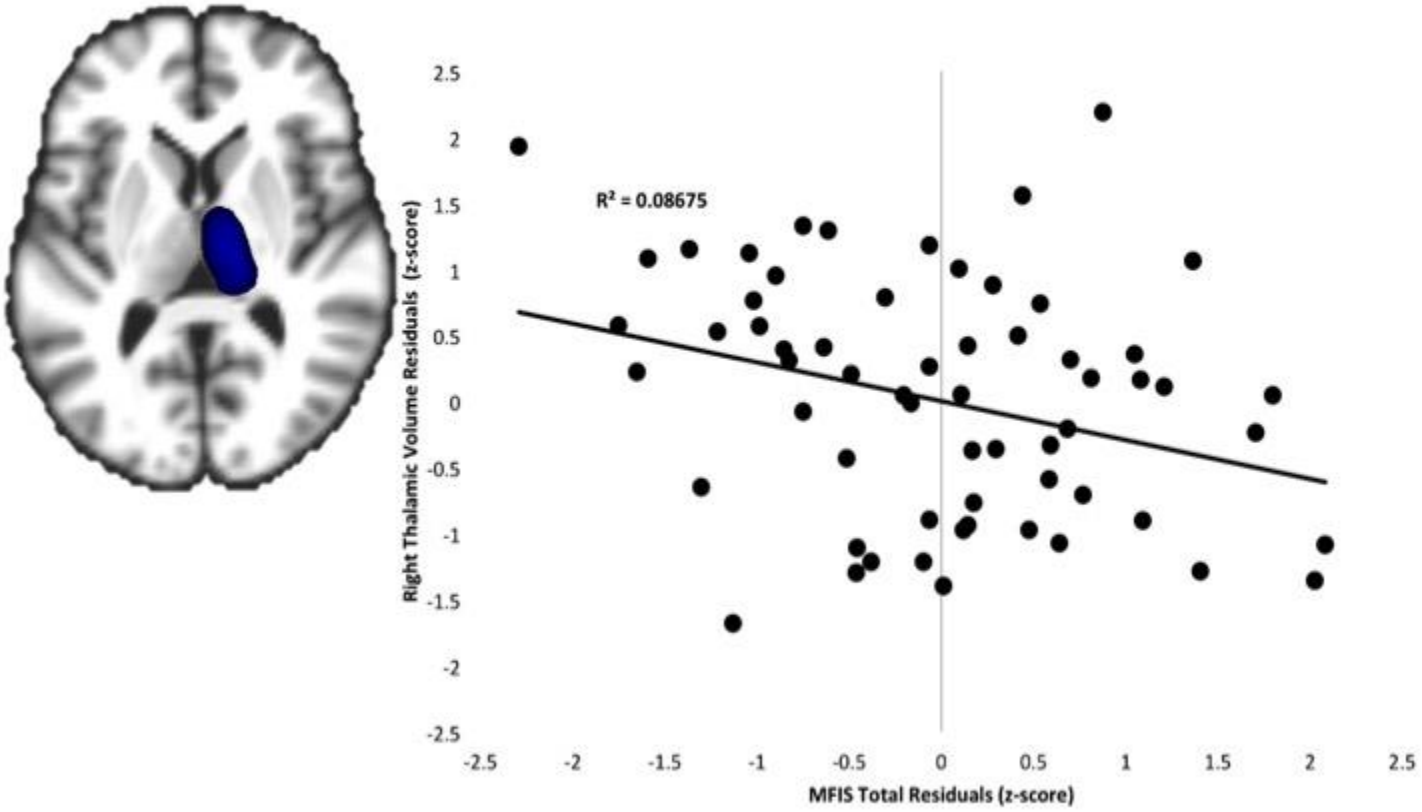
	MFIS Total Score	-12.54	6.16	-.341	.046			

Figure 1. Partial Regression Plot for Association Between Fatigue and Right Thalamic Volume



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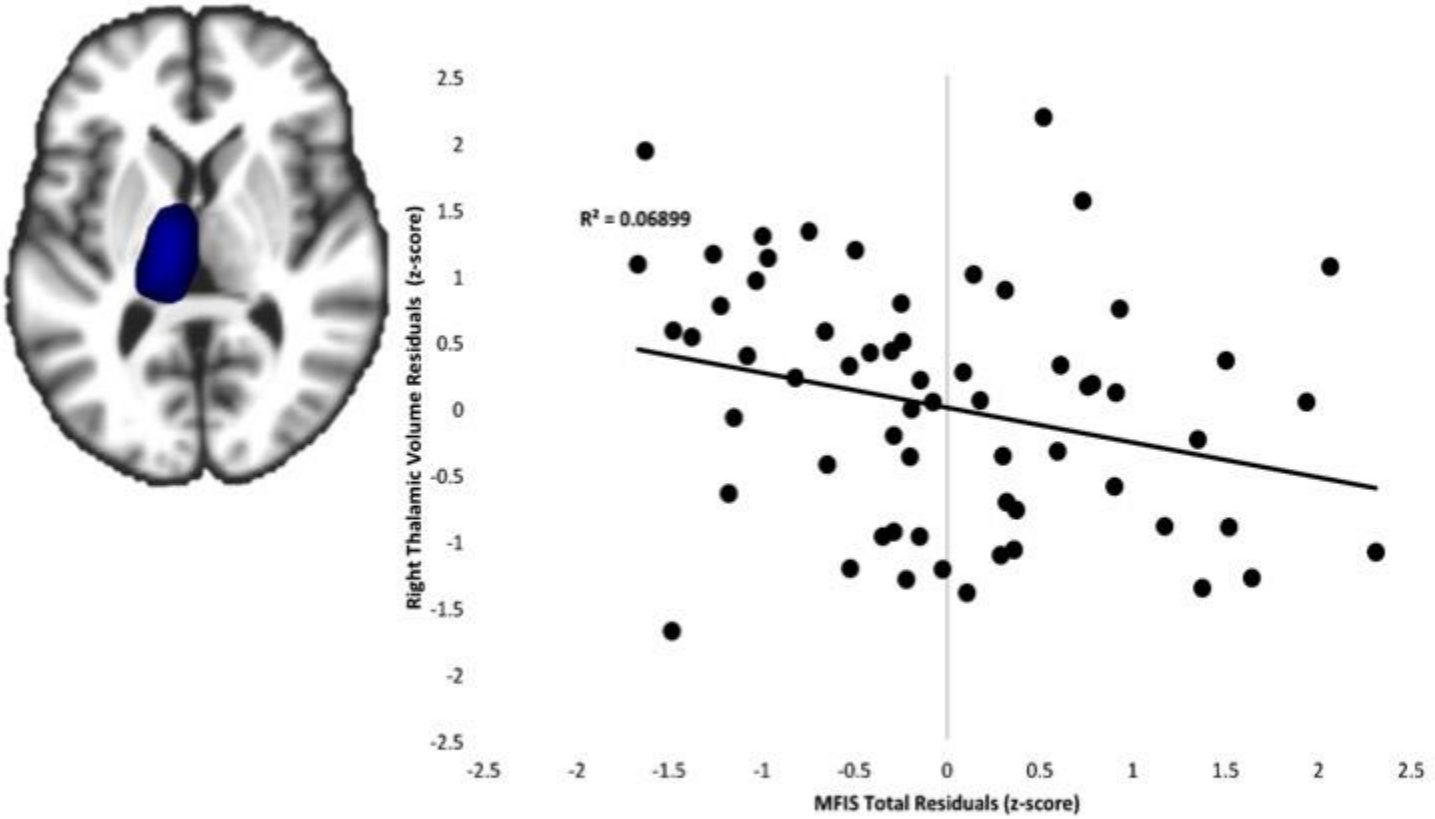
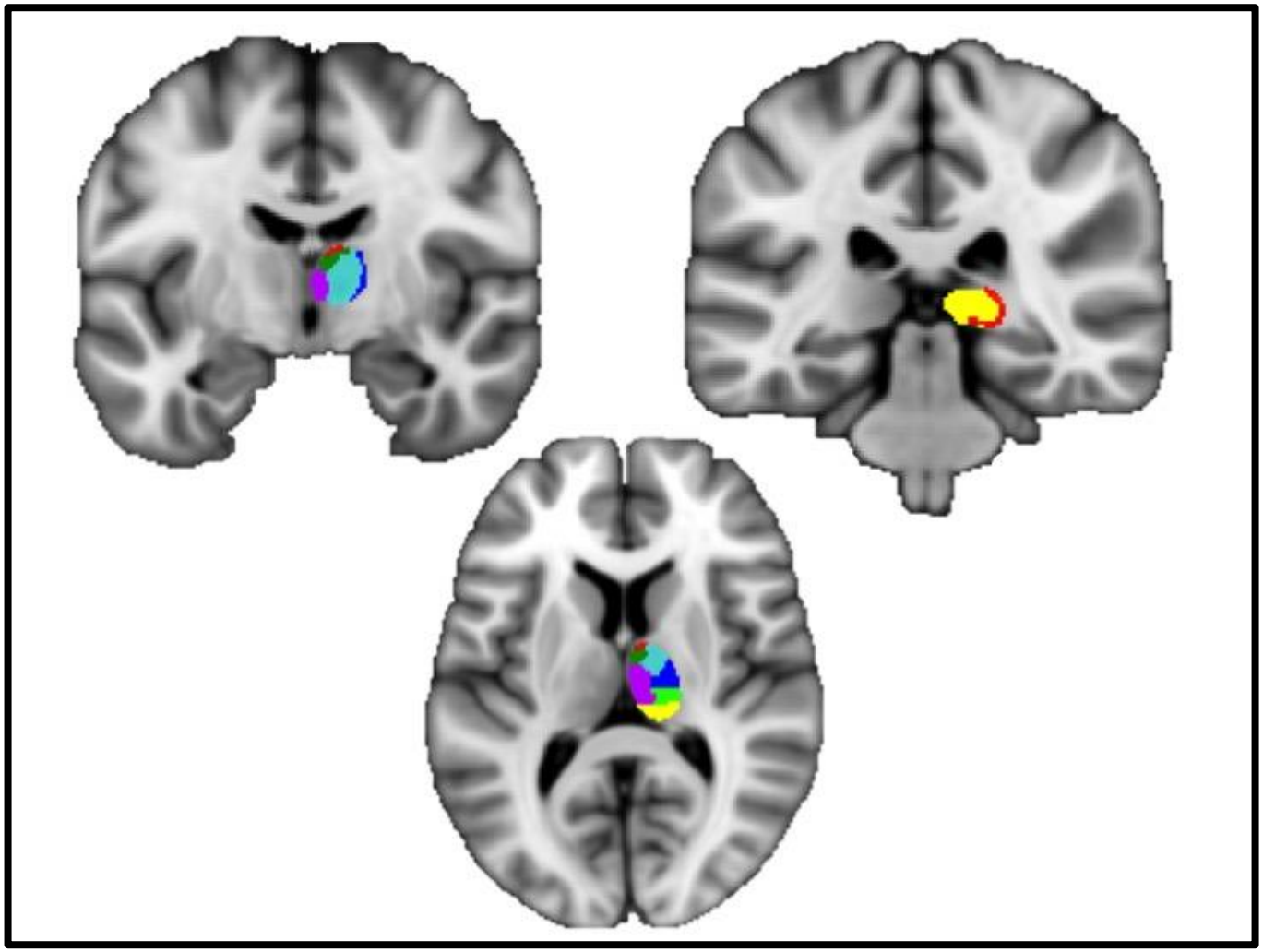


Figure 3. Regional Atrophy for Right Thalamus



Significant areas of atrophy are depicted in red ($p < .05$). Other colored areas are ICBM T1 Atlas parcellations of thalamus. Dark Green = Anterior Nucleus, Light Green = Lateral Posterior Nucleus, Dark Blue = Ventral Lateral Nucleus, Light Blue = Reticular Nucleus, Purple = Ventral Anterior Nucleus, Yellow = Dorsomedial Nucleus

Figure 4. Regional Atrophy for Left Thalamus

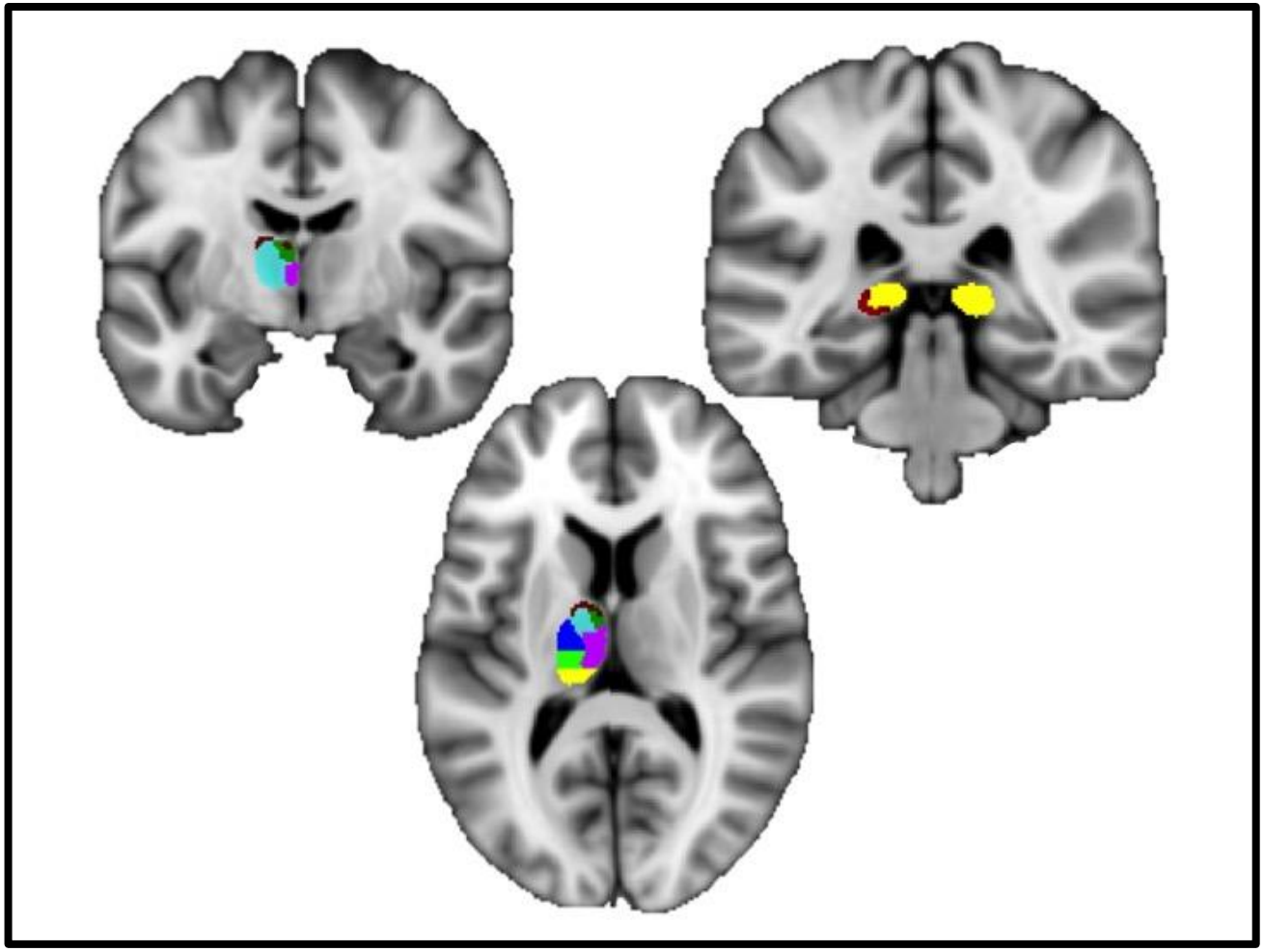


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Figure

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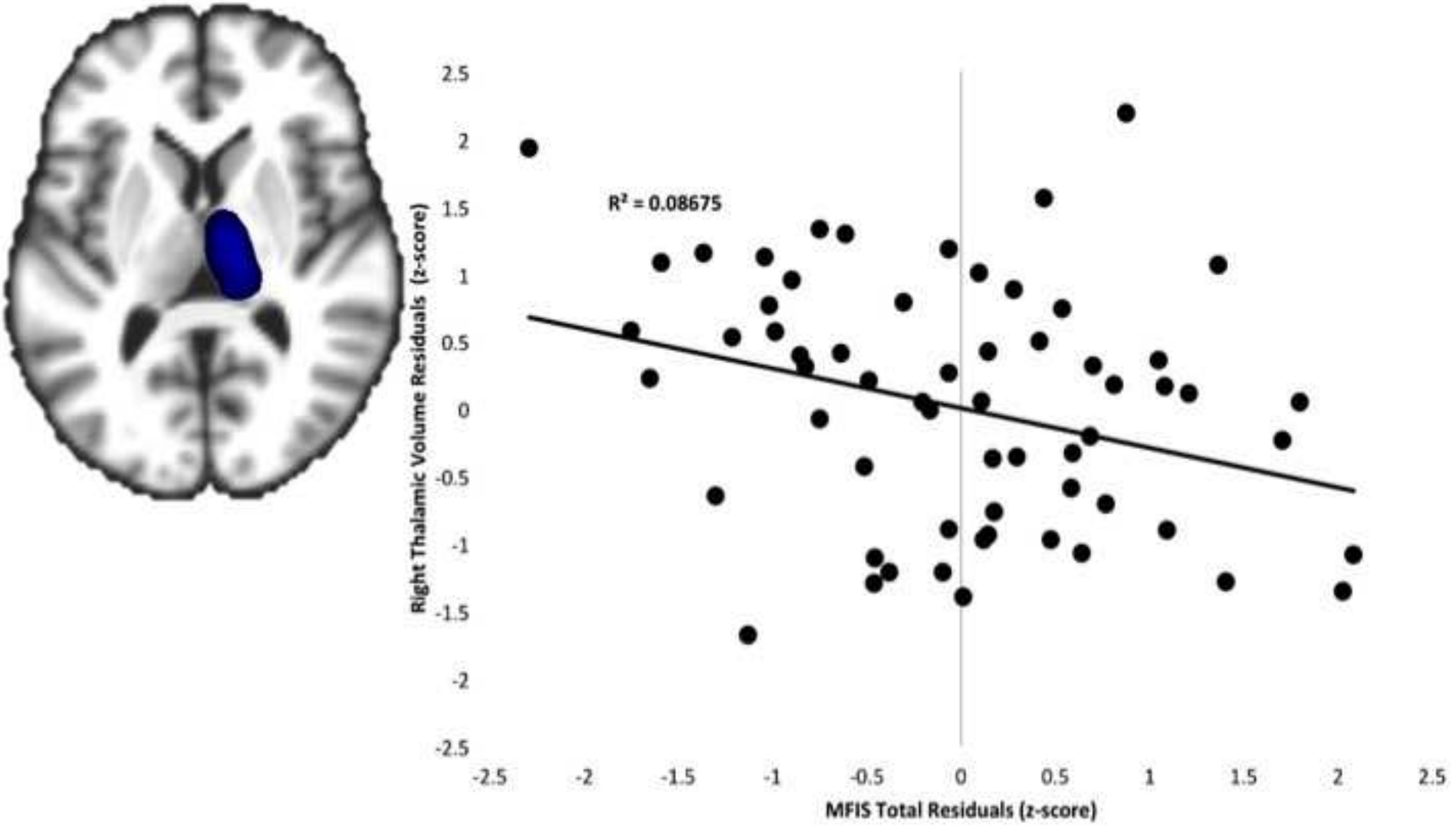

Figure 2

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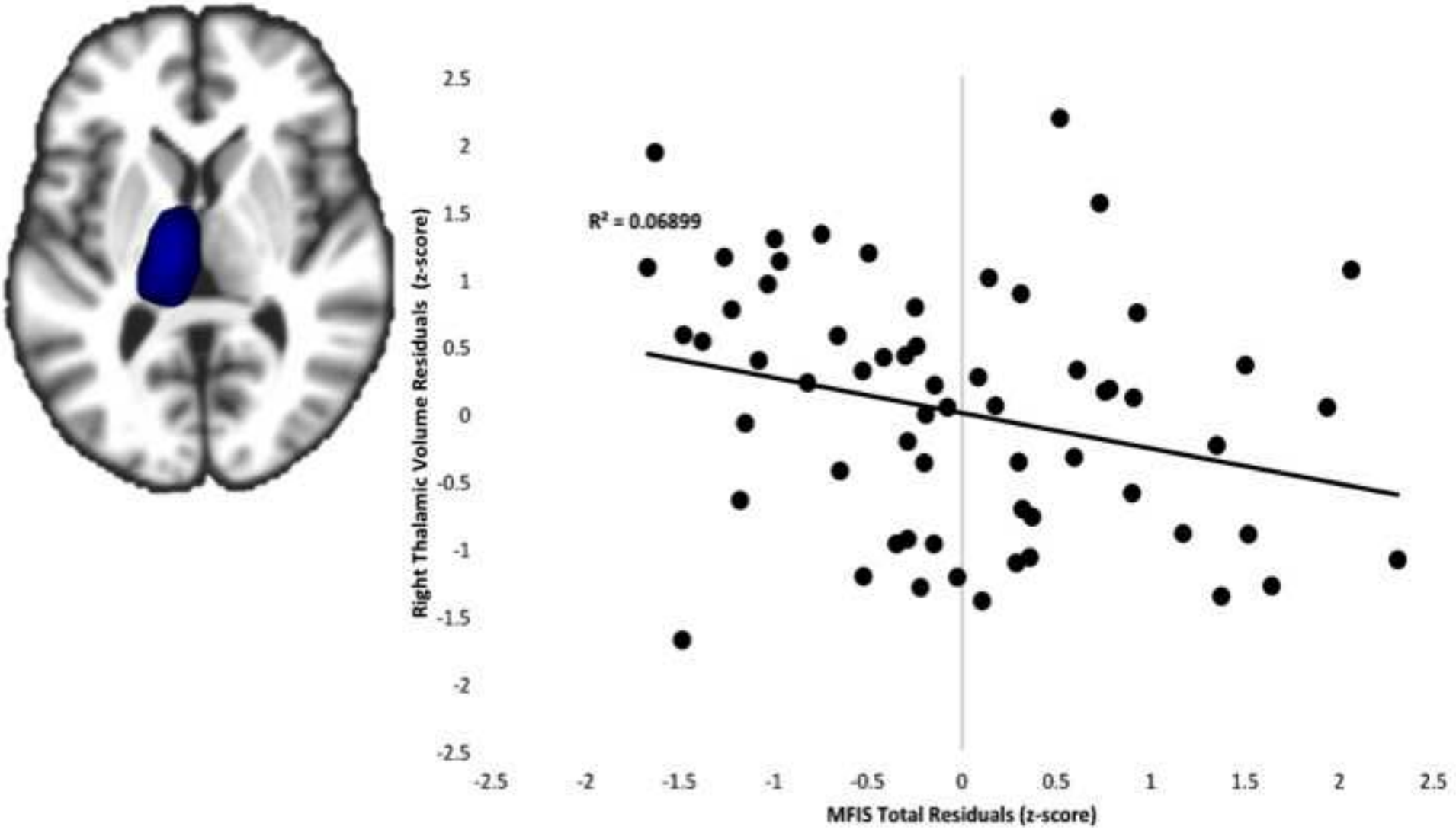

Figure 3

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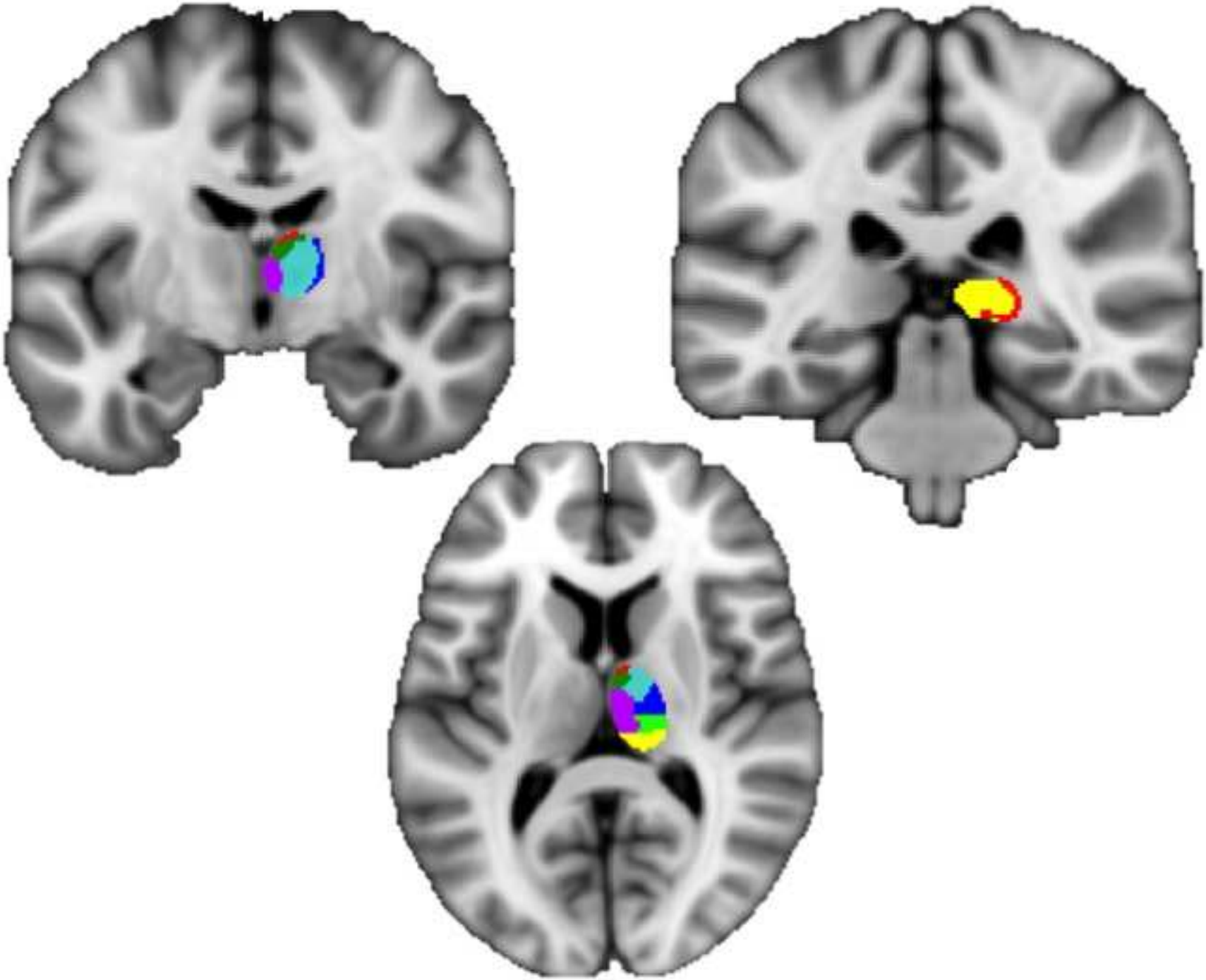

Figure 4

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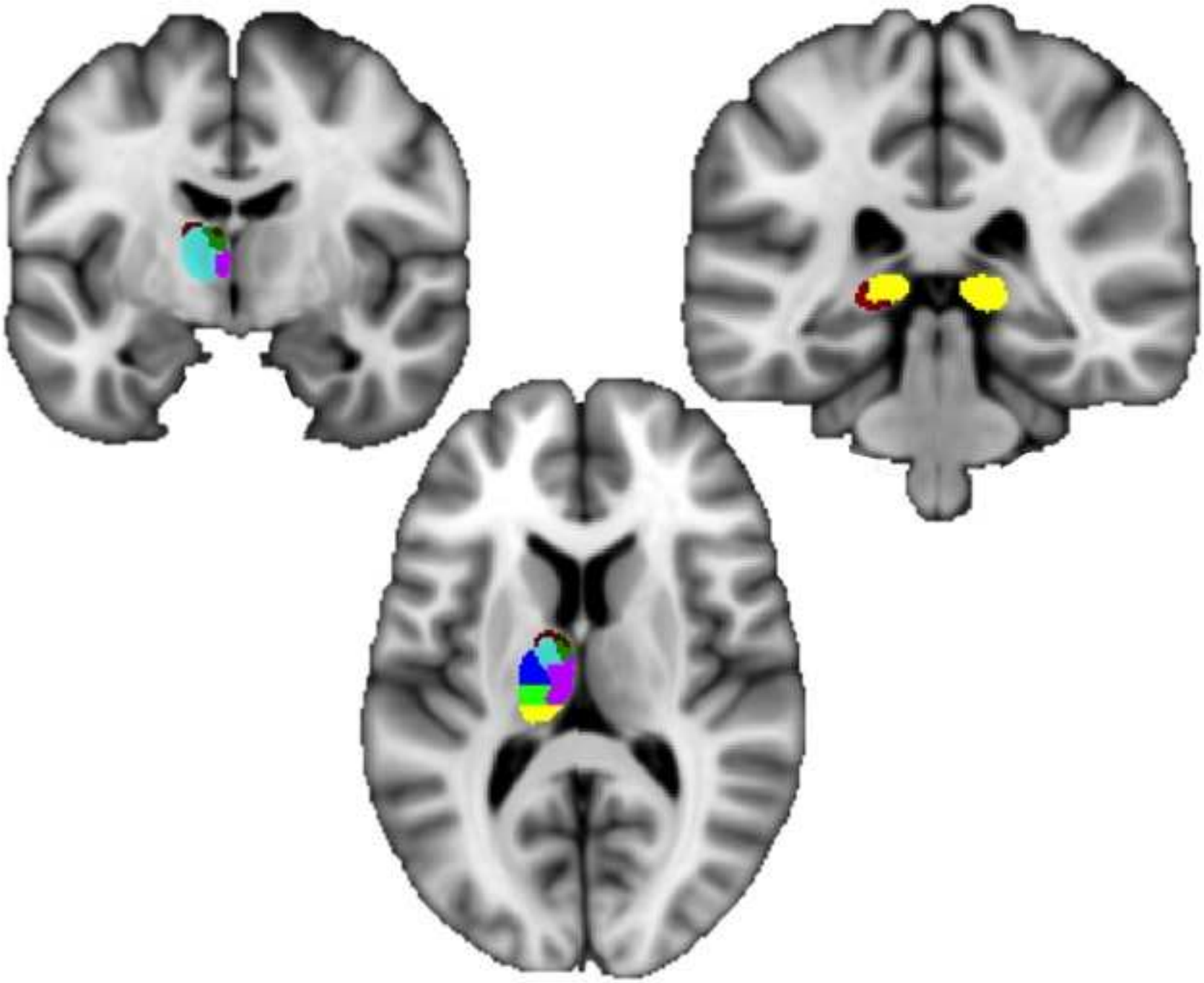

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Running head: APOE ε4 GENOTYPE & PSYCHIATRIC DISTRESS

Apolipoprotein E (APOE) ε4 Genotype is Associated with Elevated Psychiatric Distress in Veterans with a History of Mild to Moderate Traumatic Brain Injury

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Abstract

Since few studies have examined the relationship between the APOE gene and clinical outcomes following military-related traumatic brain injury (TBI), we aimed to determine whether the ε4 allele of the APOE gene influences neuropsychiatric symptoms in Veterans with a history of mild to moderate TBI. Participants included 133 Veterans (TBI=79, military controls [MC]=54) who underwent APOE genotyping and were divided into ε4+ (TBI=18, MC=15) and ε4- (TBI=61, MC=39) groups. All participants underwent evaluation of psychological distress using the Beck Depression Inventory-II (BDI-II), Beck Anxiety Inventory (BAI), and PTSD Checklist-Military Version (PCL-M). Two-way analyses of variance were conducted to examine the effect of group (TBI vs. MC) and APOE-ε4 status (ε4+ vs. ε4-) across symptom measures. There was a significant main effect of group across all symptom measures (TBI>MC; all *p*-values <.001), no main effect of ε4 genotype (*p*=.152-.222), and a significant interaction of group by ε4 genotype across all measures (*p*=.027-.047). Specifically, for TBI participants, ε4+ Veterans demonstrated significantly higher symptom scores across all measures when compared to ε4- Veterans (*p*=.007-.015). For MC participants, ε4 status had no effect on the severity of psychiatric symptom scores (*p*=.585-.708). Our results demonstrate that, in our well-characterized sample of Veterans with history of neurotrauma, possession of the ε4 allele conveys risk for increased symptomatology (i.e., depression, anxiety, PTSD) even well outside of the acute phase of injury. Findings suggest a meaningful relationship between APOE genotype and psychiatric distress following TBI, and they suggest that there is a brain basis for the complex neuropsychiatric presentation often observed in this vulnerable population. Future longitudinal studies are needed in order to further our understanding of how genetic factors influence response to TBI.

Keywords: APOE gene, genetics, traumatic brain injury, military veterans, psychiatric distress

Introduction

Research regarding clinical outcomes in the aftermath of traumatic brain injury (TBI) has burgeoned considerably, and it has become well established that military-related TBI is often coupled with high levels of psychiatric distress.[1-5] Posttraumatic stress disorder (PTSD) has undoubtedly been the most widely studied co-morbid condition associated with TBI, but other mental health diagnoses have also been linked with TBI including, but not limited to, depression and anxiety.[3; 6-9] For example, in a study of Afghanistan and Iraq-era Veterans with positive TBI screens, Carlson et al.[7] documented that over 80% of these service members also had at least one clinician-diagnosed psychiatric disorder. Interestingly, in this study, Veterans who screened positive for TBI were three times more likely to have been diagnosed with PTSD compared to those who screened negative for TBI.[7] These findings not only highlight the prevalence of psychiatric distress in this unique population, but also raise the question of why such high rates of mental health symptoms—especially PTSD—are observed in service members with a history of TBI relative to those without TBI.

A number of theories have been proposed to account for the increased rates of psychiatric distress and symptomatology in Veterans who have experienced a TBI. Notably, though, the occurrence of psychiatric distress following TBI is not specific to military-related TBI, as civilian outcome studies have also reported high rates of mental health symptoms following TBI.[10-12] Explanations for these observed comorbidities broadly fall within the realm of environmental versus biological contributions. Environmental considerations primarily relate to the context under which the TBI was sustained. For example, the presence or degree of combat exposure,[9; 13] the mechanism of injury (such as blast or blunt force),[14-16] and the severity of the injury event[14; 17] have all been hypothesized to at least partially explain the development of

persisting symptoms and/or psychiatric distress following TBI. In contrast, postulated biological mechanisms accounting for the high rates of psychiatric distress include pathophysiological changes associated with TBI, [18-20] as well as the influence of genetic predispositions.[21-23]

Our understanding of the associations between specific genetic polymorphisms and TBI susceptibility and outcome is still in its infancy. Several candidate genes have been explored thus far, but at present, the gene encoding apolipoprotein E (apoE) has been the most widely studied gene with respect to its role in recovery and outcome following TBI.[24-26] ApoE is a lipoprotein that transports and metabolizes lipids (such as cholesterol) within the central nervous system (CNS), [27; 28] and it is primarily involved in neuronal maintenance, growth, and repair.[28-30] ApoE is encoded by the apolipoprotein E (APOE) gene, located on chromosome 19, which is comprised of 3 alleles—APOE ϵ2, APOE ϵ3, and APOE ϵ4—for a total of six genotypes (three homozygous: ϵ2/ϵ2, ϵ3/ϵ3, ϵ4/ϵ4 and three heterozygous: ϵ2/ϵ3, ϵ2/ϵ4, and ϵ3/ϵ4).[31] The properties of the ϵ2, ϵ3, and ϵ4 alleles result in differential capacities for cell maintenance and repair/regrowth.[28; 32] For instance, whereas the ϵ3 allele facilitates neurite outgrowth, the ϵ4 allele inhibits neurite outgrowth.[33-35] The ϵ4 allele has also been implicated in other neuropathological processes including mitochondrial dysfunction, inflammation, increased amyloid β (Aβ) production/accumulation, and altered Aβ peptide clearance.[26; 28; 32] Thus, the ϵ4 allele is considered to be a risk factor for possible neuropathology following CNS compromise.[24; 28; 36]

The APOE gene was initially studied in the context of aging, and it has consistently been found to be a risk factor for Alzheimer's disease (AD) for a review, see Verghese et al.[37] and Kim et al.[38] More recently, there are now several lines of research suggesting that the presence of the ϵ4 allele is associated with unfavorable outcome following TBI.[23-25; 31] For example, TBI ϵ4-carriers, relative to non ϵ4-carriers, have been found to demonstrate (1) worse global and

functional outcomes; [39-43] (2) poorer neuropsychological performance post-injury;[44-47] and (3) increased risk for developing AD or other dementia.[48-50] However, despite rapid expansion of research related to the APOE gene and clinical outcome following TBI, to our knowledge, no studies have examined the relationship between the APOE gene and psychiatric symptom distress in the context of military TBI. Therefore, in the present study, we aimed to determine whether the ε4 allele of the APOE gene influences neuropsychiatric symptoms in military Veterans with and without mild to moderate TBI. We hypothesized that among Veterans with a history of TBI, ϵ4+ participants would experience greater psychiatric symptoms relative to ϵ4- participants. In contrast, we hypothesized that ϵ4 allele status would not influence psychiatric symptoms in MC's.

Materials and Methods

Participants and Procedures

Participants were 79 Veterans with a history of TBI (n=69 mild, n=10 moderate) and 54 military controls (MC) without a history of TBI who were predominantly involved in the Iraq and Afghanistan conflicts (i.e., Operation Enduring Freedom [OEF], Operation Iraqi Freedom [OIF], and Operation New Dawn [OND]). Veterans were recruited from the VA San Diego Healthcare System (VASDHS) through outpatient clinics (e.g., a TBI specialty clinic), recruitment flyers posted within the VASDHS, and word-of-mouth. Participants were administered several questionnaires, which included completion of self-report measures of psychiatric distress, as well as select modules of the Mini-International Neuropsychiatric Interview (M.I.N.I.; i.e., Major Depressive Episode and Posttraumatic Stress Disorder). The present study was reviewed and approved by local institutional review boards and informed consent was obtained from all participants prior to research participation.

An initial screening interview was conducted to determine participant eligibility. TBI history was assessed using a clinical interview adapted from the VA Semi-Structured Clinical Interview for TBI.[51] The interview was comprised of questions pertaining to the nature of previously sustained TBI's. Specifically, the following information was gathered for each TBI reported: injury-severity characteristics (presence and duration of loss of consciousness [LOC], post-traumatic amnesia [PTA], and alteration of consciousness [AOC]), the context under which the TBI was sustained (military vs. non-military event), the mechanism of injury (blast-related vs. blunt/mechanical force), and when the TBI occurred. This information was used to determine the "worst" or "most significant" TBI ever experienced by each service member, which was, in turn, used to classify injury severity for the present study. Additionally, the interview was used to

determine the total number of lifetime TBI's sustained by each participant (a TBI was counted if it met criteria as defined below).

To determine whether Veterans met criteria for having sustained a TBI, the VA/DoD Clinical Practice Guideline for Management of Concussion/Mild TBI [52] definition was applied; these guidelines indicate that in order for an event to be classified as a TBI, the individual must have experienced at least one of the following: (1) LOC, (2) AOC, (3) PTA, (4) neurological deficits (such as weakness, loss of balance, etc.), and (5) intracranial lesion.[52] For the purpose of this study, information pertaining to neurological deficits and intracranial lesions was not available for all participants; thus classification of TBI was based on participant self-reported duration of LOC, AOC, and PTA. A *mild* TBI was defined as experiencing LOC < 30 minutes, AOC up to 24 hours, and/or PTA < 24 hours; a *moderate* TBI was defined as experiencing LOC >30 minutes and <24 hours, AOC >24 hours, and/or PTA >24 hours but <7 days.[52] The interviews were conducted face-to-face by either post-baccalaureate research assistants or graduate students under the supervision of a neuropsychologist. If participants did not report a history of TBI (as defined above), these Veterans were classified as MC participants.

Exclusion criteria for the TBI group included the following: (1) history of severe TBI (defined as LOC ≥ 24 hours, AOC > 24 hours, and/or PTA ≥ 7 days); (2) having the "worst" or "most significant" TBI occur prior to the age of 18; (3) history of a neurological disorder or serious medical illness (e.g., epilepsy, multiple sclerosis, stroke, myocardial infarction, etc.); (4) history of bipolar disorder, schizophrenia, or another psychotic disorder as per *Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition, Text Revision* (DSM-IV-TR) criteria;[53] (5) current (within the past 30 days) substance/alcohol abuse or dependence as per DSM-IV-TR criteria; (6) a positive toxicology screen (measured by the Rapid Response 10-drug Test Panel);

and (7) suboptimal effort defined as a Test of Memory Malingering (TOMM)[54] score of <45 *or* a California Verbal Learning Test-II (CVLT-II)[55] Forced Choice Recognition score of <15. Exclusion criteria for the MC group included the following: (1) history of TBI (regardless of severity level) and (2) meeting criteria 3-7 as defined above for the TBI sample. Inclusion criteria for both the TBI and MC groups required that Veterans provide a DNA sample that was successfully analyzed for their APOE genotype.

Laboratory Procedures

Participants' DNA was collected via buccal sample; specifically, participants swabbed the inside of their cheek to obtain a saliva sample that could be used for APOE genotyping. The APOE genotype for each participant was determined by using two Taqman® Single Nucleotide Polymorphism (SNP) assays for the SNPs APOE112 (rs429358) and APOE158 (rs7412). Participants were genotyped using a method based on polymerase chain reaction identical to that of Saunders et al.[56] APOE genotyping results for the overall sample were as follows: ϵ2/ϵ2 (n=0, 0%), ϵ2/ϵ3 (n=12, 9.0%), ϵ2/ϵ4 (n=4, 3.0%), ϵ3/ϵ3 (n=88, 66.2%), ϵ3/ϵ4 (n=26, 19.5%), and ϵ4/ϵ4 (n=3, 2.3%). Based on these observed frequencies, participants were divided into two groups—Veterans with one or two copies of the ϵ4 allele were classified as "ϵ4 present" (ϵ4+) and Veterans with no copies of the ϵ4 allele were classified as "ϵ4 absent" (ϵ4-). Veterans were not informed of their APOE genotype.

Primary Outcome Measures

Beck Depression Inventory-II (BDI-II): The BDI-II[57] is a 21-item self-report measure assessing depressive symptomatology. Each item on the BDI-II is comprised of four statements

related to a particular symptom of depression. Participants are instructed to select the statement in each item that best describes how they have been feeling over the past two weeks. The statements correspond to values ranging from 0-3, with higher values representing more severe depressive symptomatology. A total score was calculated by adding together the ratings for the 21 items (possible range: 0-63). The psychometric properties of the BDI-II are well established.[57; 58]

Beck Anxiety Inventory (BAI): The BAI[59] is a 21-item self-report measure assessing generalized anxiety. Each item corresponds to a common symptom of anxiety and participants are asked to rate the extent to which they were bothered by each symptom during the past week using a 4-point rating scale ranging from 0 ("Not at all") to 3 ("Severely—I could barely stand it"). Higher scores represent more severe anxiety. A total score was calculated by aggregating the individual responses from each item (possible range: 0-63). Similar to the BDI-II, the BAI has sound psychometric properties.[59-62]

PTSD Checklist – Military Version (PCL-M): The PCL-M[63] is a 17-item self-report measure designed to assess DSM-IV-TR diagnostic criteria for PTSD. Each item on the PCL-M corresponds to a DSM symptom of PTSD and participants are asked to rate the extent to which they have been bothered by each symptom over the past month using a 1-5 scale, with 1 indicating "Not at all" and 5 indicating "Extremely." Higher scores represent more severe PTSD symptomatology. A total score was calculated by summing the selected values from each item (possible range: 17-85). The psychometric properties of the PCL-M have also been well established.[64-67]

Participants were also administered a measure of pre-morbid intellectual functioning—the Reading subtest of the Wide Range Achievement Test 4,[68] as well as measures of effort,

including the TOMM[54] and the CVLT-II Forced Choice Recognition subtest.[55] As noted above, participants who demonstrated performances below clinical cut-offs on one or both of these tasks were removed from the analyses.

Data Analyses

Descriptive statistics were run on the overall sample, and TBI and MC participants were compared to determine whether there were any differences between groups with regard to basic demographic characteristics. Independent-samples *t*-tests were used to evaluate continuous data and chi-square analyses were used to evaluate categorical data. Within the TBI sample, participants were divided into mild and moderate TBI groups and were compared across demographic and injury severity characteristics. TBI participants were also divided into groups based on the presence or absence of an ϵ4 allele (ϵ4+ vs. ϵ4-), and allele groups were compared across the same demographic and injury severity characteristics. Two-way analyses of variance (ANOVAs) were conducted in order to examine the effect of group (TBI vs. MC) and ε4 status (ε4+ vs. ε4-) across self-report measures of psychiatric distress. Two-way ANOVAs were also conducted to determine whether results would differ after removal of moderate TBI participants from the analyses. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 24 (SPSS IBM, New York, NY).

Results

Demographic and Injury-Related Characteristics

The overall sample included 133 military Veterans (79.7% male) who were, on average, 32.35 years old (*SD* = 7.08; *Mdn* = 30.00; range = 21-53) and who completed 14.33 years of education (*SD* = 1.74; *Mdn* = 14.00; range = 12-18). Approximately half of the participants self-identified as Caucasian (51.1%), followed by Hispanic/Latino (27.1%), African American (9.0%), Asian/Pacific Islander (8.3%) and Other (4.5%). The majority of the participants (89.5%) served in OEF/OIF/OND and 24.8% of the overall sample had at least one ϵ4 allele.

Participant demographic characteristics for the TBI (n = 79) and MC (n = 54) groups are presented in Table 1. Overall, groups were well-matched, as there were no differences between TBI and MC participants with respect to age, sex, marital status, employment status, branch of service, OEF/OIF/OND Veteran status, and APOE ϵ4 allele status. As expected, a greater proportion of participants in the TBI group (68.4%) were exposed to combat compared to the MC group (37.0%). Groups also differed on ethnicity, as 66.7% of MC's identified as Caucasian compared to 40.5% of TBI participants. Finally, education significantly differed between groups ($p = .007$), with the MC group having, on average, 0.8 more years of education than the TBI group. However, the groups did not differ on a measure of pre-morbid intellectual functioning (WRAT4 Reading subtest, $p > .05$).

Among the TBI participants, 69 (87.3%) were classified as having a mild TBI, and 10 (12.7%) were classified as having a moderate TBI. Table 2 compares TBI participants by injury severity (mild vs. moderate TBI) across demographic variables and injury-related characteristics. The average time from injury to assessment was 76.32 months (~6 years) across the TBI sample. There were no significant differences between mild and moderate TBI groups on any of the

demographic variables, and results were similar when nonparametric statistics were performed (all $p > .05$). By definition, mild and moderate groups differed on injury severity characteristics (refer to Table 2).

Table 3 compares TBI participants by ϵ4 status (ϵ4+ vs. ϵ4-) across demographic and injury-related characteristics, as well as across lifetime diagnosis of psychiatric disorders (i.e., Major Depressive Disorder, Posttraumatic Stress Disorder). Eighteen participants (22.8%) were classified as ϵ4+ and 61 (77.2%) were classified as ϵ4-. APOE ϵ4+ and ϵ4- participants did not differ across any of the demographic or injury-related characteristics, nor did the groups differ on presence of psychiatric disorders.

Psychiatric Distress

When examining the BDI-II total score, a main effect of group was found, $F(1, 129) = 59.60$, $p < .001$, $\eta_p^2 = 0.316$, such that the total score was significantly greater for TBI participants ($M = 20.89$, $SD = 12.87$) than for MC's ($M = 5.85$, $SD = 8.65$). Although the main effect of ϵ4 allele status was not significant ($F(1, 129) = 2.07$, $p = .152$, $\eta_p^2 = 0.016$), the interaction between group and ϵ4 allele status was significant ($F(1, 129) = 4.01$, $p = .047$, $\eta_p^2 = 0.030$). Specifically, as depicted in Figure 1, TBI-ϵ4+ Veterans had higher BDI-II scores than TBI-ϵ4- Veterans ($F(1, 142) = 6.77$, $p = .010$, $\eta_p^2 = 0.050$). In contrast, there were no significant group differences by APOE-ϵ4 status for MCs ($F(1, 129) = 0.14$, $p = .708$, $\eta_p^2 = 0.001$).

A main effect of group was also found for the BAI total score, $F(1, 129) = 40.43$, $p < .001$, $\eta_p^2 = 0.239$, such that BAI scores were significantly greater for TBI participants ($M = 13.43$, $SD = 11.18$) than for MC's ($M = 3.52$, $SD = 6.79$). Additionally, although there was no main effect of ϵ4 allele status ($F(1, 129) = 2.03$, $p = .157$, $\eta_p^2 = 0.015$), the interaction between

group and ϵ4 allele status was significant ($F(1, 129) = 4.99$, p = .027, $\eta_p^2 = 0.037$). For TBI participants, ϵ4+ Veterans had higher BAI scores than ϵ4- Veterans ($F(1, 129) = 7.64$, p = .007, $\eta_p^2 = 0.056$); for MC's, ϵ4 status had no effect ($F(1, 129) = 0.29$, p = .590, $\eta_p^2 = 0.002$). See Figure 2.

When examining the PCL-M total score, a main effect of group was found, $F(1, 129) = 57.43$, p < .001, $\eta_p^2 < 0.308$. Similar to the above results, the PCL-M total score was significantly greater for TBI participants ($M = 43.90$, $SD = 17.67$) than for MC's ($M = 23.65$, $SD = 11.99$). There was no main effect of ϵ4 allele status ($F(1, 129) = 1.51$, p = .222, $\eta_p^2 = 0.012$), however the interaction between group and ϵ4 allele status was significant ($F(1, 129) = 4.20$, p = .042, $\eta_p^2 = 0.032$). As seen in Figure 3, for TBI participants, ϵ4+ Veterans had higher PCL-M scores than ϵ4- Veterans ($F(1, 129) = 6.13$, p = .015, $\eta_p^2 = 0.045$); for MC's, ϵ4 status had no effect ($F(1, 129) = 0.30$, p = .585, $\eta_p^2 = 0.002$).

Importantly, removal of participants with moderate TBI from the analyses revealed the same pattern of results. Specifically, there continued to be a significant main effect of group across all symptom measures (TBI>MC; all *p*-values <.001; η_p^2=.236-.318) and no main effect of ε4 genotype (*p*=.147-.345, η_p^2=.007-.018). The group by ε4 genotype interaction remained significant for the BAI total score (*p*=.024, η_p^2=.042), and approached significance (trend) for the BDI-II total score (*p*=.058, η_p^2=.030) and PCL-M total score (*p*=.083, η_p^2=.025). APOE-ε4+ Veterans with a history of mild TBI demonstrated significantly higher symptom scores on the BAI and BDI-II when compared to ε4- Veterans with a history of mild TBI (BAI: *p*=.008, η_p^2=.058; BDI-II: *p*=.020, η_p^2=.045), and there was a trend in the same direction for the PCL-M (*p*=.054, η_p^2=.031). ε4 status had no effect on the severity of psychiatric symptom scores for MC participants (*p*=.566-.700, η_p^2=.001-.003).

Secondary Analyses

Given the observed differences in combat exposure and ethnicity between the TBI and MC samples as described above, analyses of covariance were conducted on the original sample. When controlling for combat exposure and ethnicity, a similar pattern of results was again observed—for TBI participants, ε4+ Veterans demonstrated significantly higher symptom scores across all measures when compared to ε4- Veterans (p=.008-.015); for MC participants, ε4 status had no effect on the severity of psychiatric symptom scores (p=.488-.682).

Discussion

To our knowledge, this study represents the first to explore associations between APOE genotype status and neuropsychiatric symptoms in the context of military TBI. As expected, consistent with several previous studies showing increased rates of psychiatric symptoms in military service members with head injury histories,[1; 3; 4; 7] our results showed that Veterans with a reported history of TBI endorsed greater symptoms of depression, anxiety, and PTSD relative to MC's. Additionally, although a main effect was not found for ϵ4 genotype across Veterans, a TBI by APOE-ϵ4 interaction was demonstrated, such that those with history of TBI and APOE-ϵ4 positivity showed the greatest level of psychiatric symptomatology across all measures administered. That is, among Veterans with a history of neurotrauma, greater levels of anxiety, depression, and PTSD symptoms were found in those with an ϵ4 allele relative to those without an ϵ4 allele. Importantly, there was no effect of APOE-ϵ4 in those without history of TBI. Given the proposed role of the APOE gene, and its purported mechanism of action following CNS insult,[28; 32] these findings bolster the hypothesis that, in the aftermath of neurological insult (e.g., TBI), those with an ϵ4 allele are at risk for more adverse outcomes relative to ϵ4- individuals.[24; 25; 31]

Although to our knowledge the current study represents the first to examine the relationship between the APOE gene and neuropsychiatric sequelae in Veterans with TBI, Lyons et al.[69] demonstrated that those with a higher level of combat exposure and APOE-ϵ4 genotype status were at greatest risk for experiencing symptoms of PTSD. More recently, Kimbrel et al.[70] found that the influence of the ϵ4 allele differed by level of combat exposure, such that those who experienced high levels of combat and ϵ4-positivity (1) were more likely to be diagnosed with PTSD and (2) demonstrated more severe PTSD symptoms. Interestingly, when combat

exposure was low, the ϵ4 allele had no effect on psychiatric outcome. Although these findings only held in non-Hispanic Blacks as compared to non-Hispanic Caucasians, the overall findings from Lyons et al.[69] and Kimbrel et al.[70] are generally consistent with the results of our study showing that ϵ4 genotype in the context of neurotrauma (e.g., TBI or combat exposure) may predispose individuals to increased psychiatric distress (e.g., PTSD, depression, anxiety, etc.).

Beyond establishing a specific relationship between the presence of the ϵ4 allele and neuropsychiatric sequelae, the results from the present study also lend support to the broader theory that genetic factors influence psychiatric distress following TBI. However, it is likely that still other biological and environmental contributions may impact the development of symptoms in this population.[71; 72] For instance, pre-morbid levels of psychiatric distress,[14; 73] degree of resilience,[73; 74] personality factors,[75-77] and intellectual functioning or cognitive reserve[78; 79] may also be important moderators or mediators in this relationship. Importantly, our results show that ϵ4+ and ϵ4- participants with TBI did not differ from each other with respect to either lifetime diagnosis of depression (i.e., Major Depressive Disorder) or PTSD. Thus, our finding of an association between greater levels of self-reported psychiatric symptomatology in TBI Veterans with head injury histories was not merely driven by greater overall psychiatric distress reflected by psychiatric disorder diagnoses in the ϵ4 group. The relationship between TBI and the emergence of psychiatric distress after brain injury is likely considerably complex, and the present findings provide a first attempt to better understand and elucidate a particular susceptibility gene that may be important in this multifaceted relationship.

Although speculative, there are several potential neurobiological or brain-based mechanisms that could explain the observed interaction between neurotrauma and APOE-ϵ4 genotype on psychiatric distress. One possibility is that the ϵ4 allele may exert many of its effects

through frontal subcortical regions, which are often impacted following TBI and are heavily involved in affective states, emotion regulation, and psychiatric distress.[20] Additionally, associations between APOE ϵ4 genotype and vascular risk factors on the development of depression have been proposed, such that ϵ4 positivity enhances risk for vascular disease, thereby increasing risk for depression (for a review, see Panza et al.[80]). Another consideration may be that there is a link between early neurodegenerative processes and APOE ϵ4 status (e.g., promoting deposition of abnormal amyloid and tau protein species) that influences neuropsychiatric distress through limbic insult. While additional brain-based mechanisms may also be at play, these theories may help to explain why we observe increased rates of psychiatric distress in many Veterans with a history of neurotrauma relative to those without trauma histories. We are pursuing these questions through ongoing research in our laboratory.

Taken together, results of our study show compelling evidence that APOE-e4 positivity may be more deleterious for those who have experienced neurotrauma versus those without TBI histories. Study strengths include a relatively large, well-characterized sample of Veterans with a history of TBI, examination of mental health symptoms beyond PTSD to better assess the magnitude of psychiatric distress in Veterans, and examination of participants who are well outside of the acute stage of injury to increase understanding of the long-term effects of APOE polymorphisms on TBI outcome. However, there are some limitations of the study that should be noted. First, although our male/female ratios are higher than many existing military TBI studies, the majority of participants were male Veterans (~80%), thus reducing the generalizability to females. We also examined milder forms of head injury and it is therefore unclear whether similar findings would be observed in individuals with more severe TBI. Additionally, longitudinal studies are needed in order to improve our understanding of the timeline associated

with the detrimental effects of the ϵ4 allele (i.e., how soon after injury and for how long after injury are these effects present?). Moreover, we relied on participants' self-report to characterize TBI severity and history (e.g., timing of injuries, number of injuries sustained, etc.). A final limitation is our relatively small sample size; however, the number of participants analyzed in this study is comparable—and in some cases larger than—previously published studies examining the effect of APOE genotype in the context of TBI.[44-46, 81] Nevertheless, it will be important for these findings to be replicated using larger samples. Relatedly, given the low number of participants who were homozygous for the ϵ4 allele (in the overall sample of 133, only 3 participants were ϵ4/ϵ4), concordant with its low frequency in the population, we were not able to assess a dose-response type of influence of the ϵ4 allele, although this is a future direction for our research.

In conclusion, a growing body of literature has emerged over the past several years that has established an association between TBI and symptoms of psychiatric distress. Although many studies have begun to investigate potential etiologies of this relationship, few studies have examined the relationship between genetics and the development or maintenance of neuropsychiatric sequelae following TBI. This study is the first to show that the APOE-ϵ4 allele is a risk factor for the development of neuropsychiatric symptoms in Veterans with a history of TBI. Importantly, these findings do not appear to be due to fundamental differences in demographic or injury severity characteristics, as groups were equivalent across these variables. Moreover, findings held even after removing Veterans with moderate TBI from the analyses. Although the present study furthers our understanding of how the APOE ϵ4 allele contributes to the emergence of mental health symptoms following TBI, future longitudinal studies are

necessary in order to more fully understand the association of APOE genotype status and psychiatric distress in the context of head injury.

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Author Disclosure Statement

No competing financial interests exist.

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Table 1. Demographic characteristics (N=133).

Variables	TBI Group (n=79)		MC Group (n=54)		
	M	**SD**	**M**	**SD**	**p[a]**
Age	32.43	7.05	32.22	7.19	.869
Education (years)	13.99	1.57	14.83	1.86	**.007**
WRAT4 Reading SS	100.96	10.92	103.28	9.95	.218
	N	**%**	**N**	**%**	**p[b]**
Sex					
Male	67	84.8	39	72.2	.076
Female	12	15.2	15	27.8	
Ethnicity					
Caucasian	32	40.5	36	66.7	
Hispanic	28	35.4	8	14.8	**.002**
African American	9	11.4	3	5.6	
Asian/Pacific Islander	9	11.4	2	3.7	
Other	1	1.3	5	9.3	
Married/Cohabitating					
Yes	29	36.7	21	38.9	.887
No	48	60.8	33	61.1	
Missing	2	2.5	0	0	
Currently Employed					
Yes	36	45.6	31	57.4	.204
No	42	53.2	23	42.6	
Missing	1	1.3	0	0	

Branch of Service					
Air Force	6	7.6	5	9.3	
Army	19	24.1	10	18.5	
Marines	28	35.4	16	29.6	.731
Navy	25	31.6	21	38.9	
Other	1	1.3	0	0	
Missing	0	0	2	3.7	
OEF/OIF/OND Veteran					
Yes	74	93.7	45	83.3	.056
No	5	6.3	9	16.7	
Combat Exposure					
Yes	54	68.4	20	37.0	**.002**
No	25	31.6	30	55.6	
Missing	0	0	4	7.4	
APOE ϵ4 allele Status					
ϵ4 Present (ϵ4+)	18	22.8	15	27.8	.513
ϵ4 Absent (ϵ4-)	61	77.2	39	72.2	

Abbreviations: WRAT4 = Wide Range Achievement Test 4; SS = standard score; OEF = Operation Enduring Freedom; OIF = Operation Iraqi Freedom; OND = Operation New Dawn. [a]Independent samples *t*-tests were used to determine whether there were group differences for age, education, and WRAT4 Reading. [b]Chi-square analyses were used to determine whether there were group differences for sex, ethnicity, marital status, employment status, military branch of service, OEF/OIF/OND status, combat exposure, and APOE ϵ4 allele status.

Table 2. TBI participants: Sample characteristics by TBI severity (N=79).

Variables	Mild TBI Group (n=69)		Moderate TBI Group (n=10)		
	M	**SD**	**M**	**SD**	**p[a]**
Age	32.00	7.02	35.40	6.88	.155
Education (years)	13.91	1.61	14.50	1.18	.270
WRAT4 Reading SS	100.75	11.19	102.40	9.28	.658
Time (months) from most recent TBI to testing	65.13	48.96	64.50	46.50	.970
Time (months) from most sig. TBI to testing	75.56	51.75	81.50	57.70	.739
Age at most sig. TBI	26.06	6.16	28.50	6.85	.251
Lifetime number of TBI's	2.36	1.33	2.50	1.72	.769
	N	**%**	**N**	**%**	**p[b]**
Sex					
Male	59	85.5	8	80.0	.650
Female	10	14.5	2	20.0	
Ethnicity					
Caucasian	29	42.0	3	30.0	
Hispanic	24	34.8	4	40.0	
African American	9	13.0	0	0	.257
Asian/Pacific Islander	6	8.7	3	30.0	
Other	1	1.4	0	0	
Married/Cohabitating					
Yes	28	40.6	1	10.0	.053

No	39	56.5	9	90.0	
Missing	2	2.9	0	10.0	
Currently Employed					
Yes	30	43.5	6	60.0	.347
No	38	55.1	4	40.0	
Missing	1	1.4	0	0	
Branch of Service					
Air Force	5	7.2	1	10.0	
Army	18	26.1	1	10.0	.647
Marines	25	36.2	3	30.0	
Navy	20	29.0	5	50.0	
Other	1	1.4	0	0	
OEF/OIF/OND Veteran					
Yes	66	95.7	8	80.0	.057
No	3	4.3	2	20.0	
Combat Exposure					
Yes	49	71.0	5	50.0	.182
No	20	29.0	5	50.0	
LOC (most sig. TBI)					
Yes	39	56.5	10	100.0	**.008**
No	30	43.5	0	0	
AOC (most sig. TBI)					
Yes	30	43.5	0	0	**.008**
No	39	56.5	10	100.0	
PTA (most sig. TBI)					**.009**

Yes	33	47.8	10	100.0	
No	30	43.5	0	0	
Unsure	5	7.2	0	0	
Missing	1	1.4	0	0	
APOE ϵ4 allele Status					
ϵ4 Present (ϵ4+)	15	21.7	3	30.0	.561
ϵ4 Absent (ϵ4-)	54	78.3	7	70.0	

Abbreviations: WRAT4 = Wide Range Achievement Test 4; SS = standard score; TBI = traumatic brain injury; sig. = significant; OEF = Operation Enduring Freedom; OIF = Operation Iraqi Freedom; OND = Operation New Dawn; LOC = loss of consciousness; AOC = alteration of consciousness; PTA = post-traumatic amnesia. [a]Independent samples *t*-tests were used to determine whether there were group differences for age, education, WRAT4 Reading, time from most recent TBI to testing, time from most significant TBI to testing, age at most significant TBI, and lifetime number of TBI's. [b]Chi-square analyses were used to determine whether there were group differences for sex, ethnicity, marital status, employment status, military branch of service, OEF/OIF/OND status, combat exposure, presence of LOC, presence of AOC, presence of PTA, and APOE ϵ4 allele status.

Table 3. TBI participants: Sample characteristics by ϵ4 allele group (N=79).

Variables	ε4 allele Present (ε4+) (n=18)		ε4 allele Absent (ε4-) (n=61)		
	M	**SD**	**M**	**SD**	**p[a]**
Age	33.56	7.94	32.10	6.81	.445
Education (years)	13.50	1.30	14.13	1.62	.134
WRAT4 Reading SS	100.17	8.42	101.20	11.63	.727
Months since most recent TBI	56.72	37.96	67.55	51.08	.408
Months since most sig. TBI	67.11	42.48	79.08	54.78	.397
Age at most sig. TBI	28.22	7.35	25.82	5.86	.154
Number of TBI's sustained	2.17	1.34	2.44	1.39	.457
	N	**%**	**N**	**%**	**p[b]**
Sex					
Male	15	83.3	52	85.2	.843
Female	3	16.7	9	14.8	
Ethnicity					
Caucasian	6	33.3	26	42.6	.439
Hispanic	7	38.9	21	34.4	
African American	4	22.2	5	8.2	
Asian/Pacific Islander	1	5.6	8	13.1	
Other	0	0	1	1.6	
Married/Cohabitating					
Yes	6	33.3	23	37.7	.819
No	11	61.1	37	60.7	

Missing	1	5.6	1	1.6	
Currently Employed					
Yes	7	38.9	29	47.5	.481
No	11	61.1	31	50.8	
Missing	0	0	1	1.6	
Branch of Service					
Air Force	0	0	6	9.8	.277
Army	3	16.7	16	26.2	
Marines	6	33.3	22	36.1	
Navy	9	50.0	16	26.2	
Other	0	0	1	1.6	
OEF/OIF/OND Veteran					
Yes	18	100.0	56	91.8	.209
No	0	0	5	8.2	
Combat Exposure					
Yes	10	55.6	44	72.1	.184
No	8	44.4	17	27.9	
TBI Severity					
Mild	15	83.3	54	88.5	.561
Moderate	3	16.7	7	11.5	
LOC (most sig. TBI)					
Yes	9	50.0	40	65.6	.232
No	9	50.0	21	34.4	
AOC (most sig. TBI)					
Yes	9	50.0	21	34.4	.232

No	9	50.0	40	65.5	
PTA (most sig. TBI)					
Yes	10	55.6	33	54.1	
No	8	44.4	22	36.1	.423
Unsure	0	0	5	8.2	
Missing	0	0	1	1.6	
Lifetime Diagnosis: MDD[c]					
Yes	8	50.0	20	39.2	.445
No	8	50.0	31	60.8	
Lifetime Diagnosis: PTSD[c]					
Yes	8	53.3	23	45.1	.574
No	7	46.7	28	54.9	

Abbreviations: WRAT4 = Wide Range Achievement Test 4; SS = standard score; TBI = traumatic brain injury; sig. = significant; OEF = Operation Enduring Freedom; OIF = Operation Iraqi Freedom; OND = Operation New Dawn; LOC = loss of consciousness; AOC = alteration of consciousness; PTA = post-traumatic amnesia. MDD = Major Depressive Disorder; PTSD = post-traumatic stress disorder. [a]Independent samples *t*-tests were used to determine whether there were group differences for age, education, WRAT4 Reading, time from most recent TBI to testing, time from most significant TBI to testing, age at most significant TBI, and lifetime number of TBI's. [b]Chi-square analyses were used to determine whether there were group differences for sex, ethnicity, marital status, employment status, military branch of service, OEF/OIF/OND status, combat exposure, TBI severity, presence of LOC, presence of AOC, presence of PTA, lifetime diagnosis of MDD, and lifetime diagnosis of PTSD. Lifetime diagnosis of MDD and PTSD were gathered through one-on-one interview using the Mini-

International Neuropsychiatric Interview (M.I.N.I.; Sheehan et al., 2006). Furthermore, M.I.N.I. data were not available for all participants; MDD diagnostic information was available for 67 of the 79 TBI participants, and PTSD diagnostic information was available for 66 of the 79 TBI participants.

Figure Legends

Figure 1. BDI-II total score across traumatic brain injury (TBI) and military control (MC) participants by Ɛ4 genotype. Mean scores with standard errors are displayed.

Figure 2. BAI total score across traumatic brain injury (TBI) and military control (MC) participants by Ɛ4 genotype. Mean scores with standard errors are displayed.

Figure 3. PCL-M total score across traumatic brain injury (TBI) and military control (MC) participants by Ɛ4 genotype. Mean scores with standard errors are displayed.

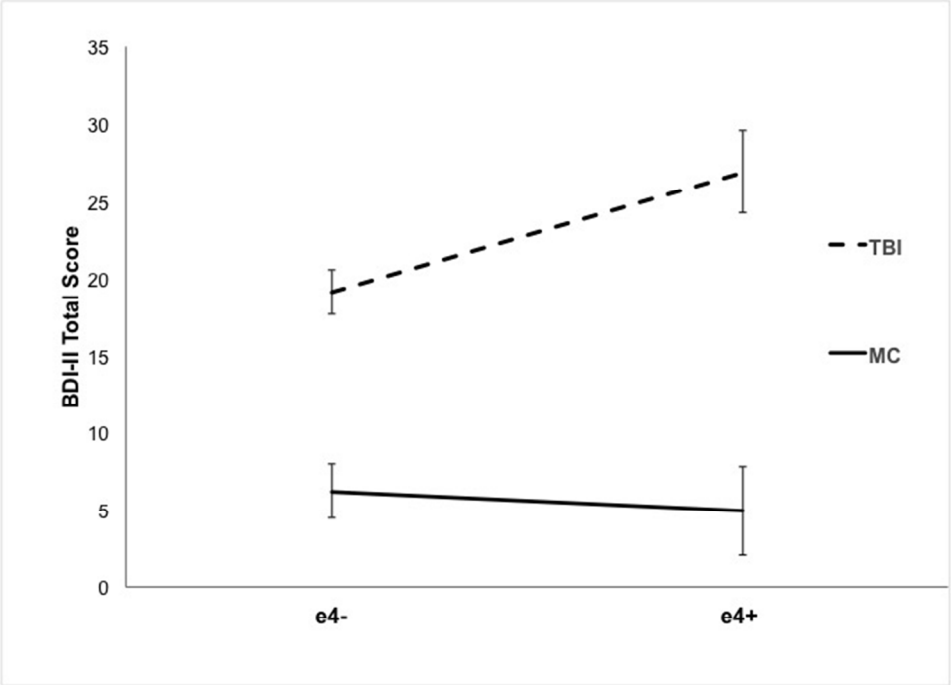

Figure 1. BDI-II total score across traumatic brain injury (TBI) and military control (MC) participants by ε4 genotype. Mean scores with standard errors are displayed.

254x190mm (72 x 72 DPI)

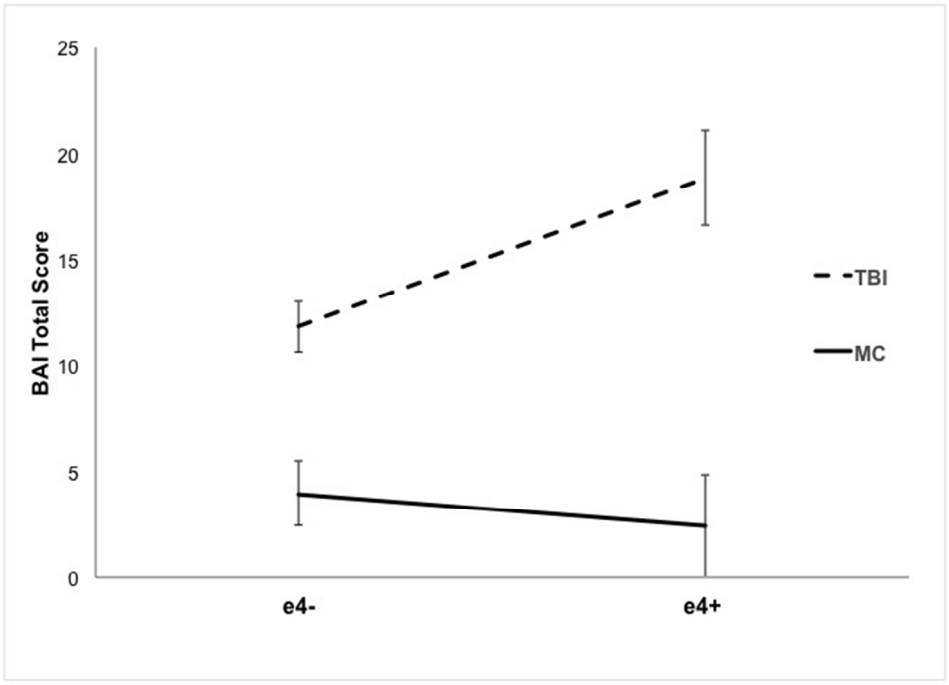

Figure 2. BAI total score across traumatic brain injury (TBI) and military control (MC) participants by ε4 genotype. Mean scores with standard errors are displayed.

254x190mm (72 x 72 DPI)

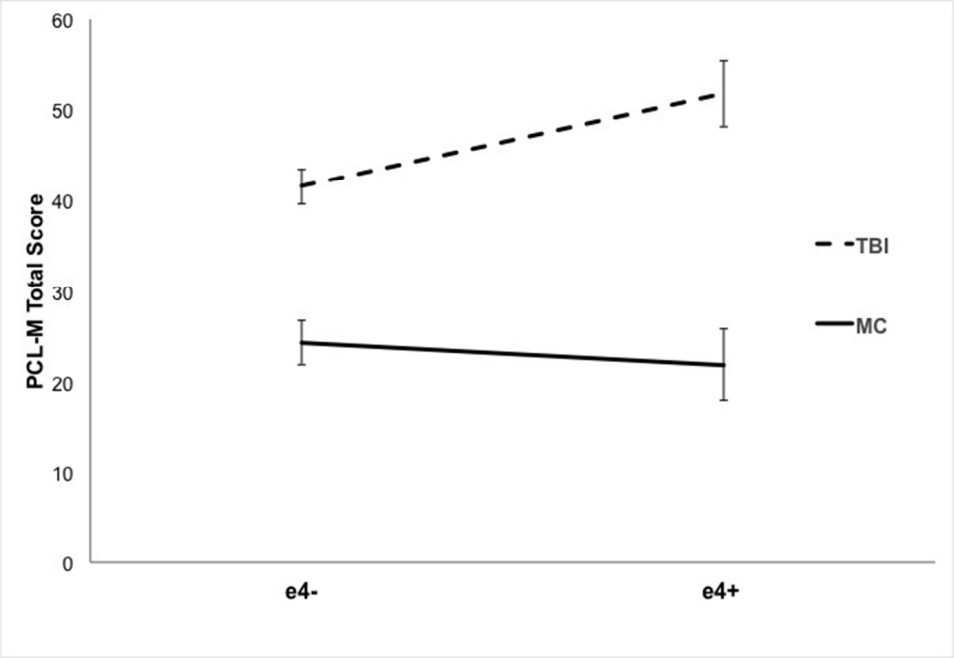

Figure 3. PCL-M total score across traumatic brain injury (TBI) and military control (MC) participants by ε4 genotype. Mean scores with standard errors are displayed.

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Running head: APOE & NEUROPSYCHOLOGICAL PERFORMANCE

Apolipoprotein E (APOE) ε4 Genotype is Associated with Reduced Neuropsychological Performance in Military Veterans with Post-Acute Mild Traumatic Brain Injury

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ABSTRACT

Objective: The purpose of this study was to investigate the effect of the APOE ε4 allele on neuropsychological functioning in military Veterans with post-acute (>1 year since injury) mild traumatic brain injury (mTBI). **Method:** Participants were 99 Veterans (mTBI=53; military controls [MC]=46) who underwent neuropsychological assessment and APOE genotyping. Three neurocognitive composite scores—memory (α=.84), speed (α=.85), and executive functioning (α=.76)—were computed from 24 norm-referenced variables, and the total number of impaired scores (>1.5 *SD* below mean) for each participant was calculated. Primary analyses included two-way ANOVAs to examine the effect of group (mTBI vs. MC) and ε4 status (ε4+ vs. ε4-) on cognition. **Results:** Collapsed across the sample, there was a significant main effect of ε4 status for the memory and speed composites (ε4+<ε4-; p=.005-.040), as well as the total number of impaired scores (ε4+>ε4-; p=.017). When evaluating only those with mTBI, the ε4+ group performed more poorly than the ε4- group on the memory (p=.048) and speed (p=.018) composites, and displayed a significantly greater number of impaired scores (p=.017), even after adjusting for PTSD symptoms. In contrast, there were no significant differences across any of the cognitive variables between ε4+ and ε4- MC's (all p>.05). **Conclusions:** Results suggest that in Veterans with post-acute mTBI, APOE-ε4 genotype is related to reduced memory and processing speed performance, as well as overall cognitive impairment. Future longitudinal work is needed to elucidate the underlying brain-based mechanisms of ε4 allelic effects on cognitive and clinical outcomes following TBI.

Keywords: APOE gene, mild traumatic brain injury, neuropsychological assessment, military veterans, cognition

Public Significance Statement: This study demonstrates that possession of a particular genetic variant (the APOE ε4 allele) negatively influences cognitive functioning in military Veterans with a history of mild traumatic brain injury (TBI). In comparison, the ε4 allele does not appear to have the same negative influence on cognitive functioning in Veterans without a history of TBI, suggesting that it is the combination of TBI and ϵ4-positivity that increases the likelihood of cognitive dysfunction.

INTRODUCTION

Recovery patterns and clinical outcomes following mild traumatic brain injury (mTBI) are widely diverse and person-specific. While no two brain injuries are identical, it is common for individuals to experience cognitive difficulties with processing speed, attention, and memory in the aftermath of sustaining an mTBI (Belanger, Curtiss, Demery, Lebowitz, & Vanderploeg, 2005; Dolan et al., 2012; Frencham, Fox, & Maybery, 2005). These cognitive deficits are generally present during the acute phase of injury, with some studies showing full recovery occurring anywhere from 1-2 weeks to 3 months after injury (Belanger et al., 2005; Binder, Rohling, & Larrabee, 1997; McCrea et al., 2009; Schretlen & Shapiro, 2003). In some cases, though, cognitive complaints and even objective impairment on neuropsychological tests persist well beyond the acute phase of injury (Clark et al., 2016; Dikmen, Machamer, Fann, & Temkin, 2010; Jak et al., 2015; Sorg et al., 2016; Tanielian & Jaycox, 2008).

Several factors have been shown to influence cognitive recovery following mTBI, including demographic, psychosocial, premorbid intellectual, and injury-related variables (Belanger et al., 2005; Drag, Spencer, Walker, Pangilinan, & Bieliauskas, 2012; McCauley et al., 2013; Ponsford, 2013; Ponsford et al., 2000). Within the context of military mTBI, recovery is often further complicated by additional factors such as combat stress/PTSD, chronic pain, and substance use (Belanger, Donnell, & Vanderploeg, 2014; Combs et al., 2015; Cooper, Vanderploeg, Armistead-Jehle, Lewis, & Bowles, 2014; Iverson, Langlois, McCrea, & Kelly, 2009; Lange et al., 2014). Emerging evidence suggests that genetic polymorphisms may also play a unique role in the recovery process, and they may help to explain the heterogeneous outcomes that are often observed following brain injury (for a review, see Dardiotis et al., 2010; Jordan, 2007; McAllister, 2011; Wilson & Montgomery, 2007). However, despite the growth in

genetic and genomic research, the influence of such factors on neuropsychological performance following mTBI remains poorly understood. It would be clinically meaningful to better understand what contributes to increased risk of neurocognitive compromise in the aftermath of TBI, as these deficits likely reflect underlying brain dysfunction (Bigler & Maxwell, 2012; Eierud et al., 2014; Mayer, Bellgowan, & Hanlon, 2015), are associated with dramatically increased service utilization and costs, and can interfere with community reintegration, psychosocial functioning, and overall quality of life (Boosman et al., 2017; Dikmen, Machamer, & Temkin, 2017; Dikmen, McLean, & Temkin, 1986; Dolan et al., 2012; Martindale et al., 2016).

To date, the apolipoprotein E (APOE) gene has been the most extensively studied gene within the TBI literature since it is well known to be specifically involved in neural restoration and synaptogenesis following brain injury (Mahley & Huang, 2012). The APOE gene, located on chromosome 19, serves many functions within the central nervous system (CNS), including lipid transportation and clearance, as well as maintenance of neural integrity and synapto-dendritic connections (Mahley, Nathan, & Pitas, 1996; Mahley, Weisgraber, & Huang, 2006). The APOE gene has three common allelic variations—ϵ2, ϵ3, and ϵ4—which notably differ in their capacity for stimulating neuritie outgrowth following CNS insult (Horsburgh, McCarron, White, & Nicoll, 2000; Mahley et al., 2006; Nathan, Bellosta, Weisgraber, Mahley, & Pitas, 1994). Compared to the ϵ2 and ϵ3 alleles, the ϵ4 allele is detrimental in this process, as it inhibits neurite outgrowth, disrupts neuronal cytoskeleton, and magnifies amyloid β (Aβ) accumulation (Horsburgh et al., 2000; Huang et al., 2001; Ji et al., 2002; Mahley et al., 2006), and markedly exacerbates tau-mediated neurodegeneration (Shi et al., 2017). Additionally, within the context of TBI, the ϵ4 allele has been linked with increased likelihood of cerebrovascular pathology

(Smith, Graham, Murray, Stewart, & Nicoll, 2006), larger heamatoma volumes (Liaquat, Dunn, Nicoll, Teasdale, & Norrie, 2002), and the presence of cerebral amyloid angiopathy (Leclercq et al., 2005).

With regard to the relationship between the APOE ϵ4 allele and neuropsychological outcomes following TBI, research findings have been mixed. While several studies have documented that individuals with an ϵ4 allele demonstrate poorer neuropsychological performance post-injury (Ariza et al., 2006; Crawford et al., 2002; Eramudugolla et al., 2014; Liberman, Stewart, Wesnes, & Troncoso, 2002; Merritt, Rabinowitz, & Arnett, 2017; Sundström et al., 2004), others have failed to find a detrimental effect of the ϵ4 allele on neuropsychological outcome after TBI (Chamelian, Reis, & Feinstein, 2004; Han et al., 2007; Hodgkinson, Gillett, & Simpson, 2009; Millar, Nicoll, Thornhill, Murray, & Teasdale, 2003; Padgett, Summers, Vickers, McCormack, & Skilbeck, 2016; Ponsford, Rudzki, Bailey, & Ng, 2007; Pruthi et al., 2010; Shadli, Pieter, Yaacob, & Rashid, 2011). In fact, a recent meta-analysis by Padgett et al. (2016) concluded that the ϵ4 allele does not appear to have an unfavorable effect on cognitive functioning following TBI—both in the context of global cognitive functioning, as well as specific cognitive domians. However, the authors cautioned that their findings are provisional due to the modest samples sizes utilized in previous studies, the small effect sizes that have been reported, and issues related to publication and replication biases (Padgett et al., 2016). Other major issues contributing to the mixed findings in the literature are differences in demographic and injury-related characteristics across studies. For example, interpretation of results is made especially challenging given the heterogeneity of injury differences in TBI severity (mild, moderate, severe), poor documentation of head injury severity measures among our combat Veterans, age of participants under investigation (pediatric/adolescent, adult, older adult), and

the setting under which the TBI was sustained (civilian, sports-related, military). Timing of the injury relative to the timing of the assessment is another key variable that is often not considered in previous studies. Among published studies examining the influence of the APOE gene on cognitive outcome following TBI, participants were assessed anywhere from weeks/months post-injury to decades following the injury (for a review, see Dardiotis et al., 2010; Lawrence, Comper, Hutchison, & Sharma, 2015). Finally, the outcomes of interest have varied widely across studies. Specifically, there are notable differences with respect to what measures were utilized and what constructs were compared (i.e., individual cognitive scores, neurocognitive composite scores, etc.). Taken together, the discrepant results observed in this literature may be attributable to the use of heterogeneous samples and varied methodologies across studies, making it difficult to draw any definitive conclusions about the effects of the ϵ4 allele on post-injury neuropsychological outcome.

Given the variability noted above, the primary objective of the present study was to evaluate a more homogeneous sample (well-characterized Veterans with mTBI) in order to reduce the number of confounding variables that could be contributing to the disparate results currently reported in the APOE-TBI neuropsychological literature. Our goal was to examine the influence of the ϵ4 allele on (1) mean neuropsychological performance across multiple cognitive domains and (2) the total number of impaired test scores in Veterans with mTBI who were in the post-acute (>1 year since injury) phase of injury. We hypothesized that mTBI participants with at least one copy of the APOE-ϵ4 allele would experience poorer neuropsychological functioning relative to mTBI ϵ4- participants.

METHOD

Participants and Procedures

Participants comprised 99 Veterans (n=53 mTBI, n=46 MC) who were recruited from a local Veterans Affairs Medical Center. Participants predominantly served in Operation Enduring Freedom (OEF), Operation Iraqi Freedom (OIF), and/or Operation New Dawn (OND) (87.0%). Veterans underwent a comprehensive neuropsychological assessment and were asked to provide a DNA sample (buccal swab) for APOE genotyping. Appropriate local institutional review boards reviewed and approved this study, and all Veterans signed an informed consent form prior to engaging in any research-related activities.

Exclusion criteria for the present study included: (1) history of a serious medical illness or neurological disorder (e.g., myocardial infarction, stroke, multiple sclerosis, etc.); (2) history of a severe mental illness (e.g., schizophrenia, bipolar disorder, etc.); (3) current (within the past 30 days) alcohol or substance abuse or dependence as per *Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition, Text Revision* (DSM-IV-TR) criteria (APA, 2000), or a positive toxicology screen as measured by the Rapid Response 10-Drug Test Panel; and (4) suboptimal effort[1] according to manual-defined cutoffs for the California Verbal Learning Test-II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000) Forced Choice Recognition *or* the Test of Memory Malingering (TOMM; Tombaugh, 1996). Study inclusion criteria required that Veterans (1) provide a buccal sample for APOE genotyping and (2) have a complete set of neuropsychological data (i.e., no missing data for any of the neuropsychological test indices utilized in the present study). Specific inclusion criteria for the mTBI group included (1) having

[1] Participants were excluded if they did not perform adequately on *one* or *both* of the validity indices (CVLT-II Forced Choice Recognition and TOMM Trial 2).

a history of mTBI only and (2) be in the post-acute phase of injury (as defined by the most recent mTBI occurring >1 year prior to the current assessment).

To determine participant eligibility for the study, Veterans were interviewed using a modified version of the VA Semi-Structured Clinical Interview for TBI (Vanderploeg, Groer, & Belanger, 2012). Trained research assistants or graduate students who were supervised by a study neuropsychologist conducted the in-person interviews. Information was collected regarding (1) the total number of lifetime TBI's sustained; (2) injury-severity characteristics for each TBI reported, including duration of loss of consciousness (LOC), duration of alteration of consciousness (AOC), and duration of post-traumatic amnesia (PTA); (3) mechanism(s) of injury; and (4) the date of each injury reported. The VA/DoD Clinical Practice Guideline for Management of Concussion/Mild TBI (2009) definition was utilized to determine whether Veterans met criteria for having sustained an mTBI, as defined by experiencing an LOC < 30 minutes, AOC up to 24 hours, and/or PTA < 24 hours. Veterans who reported at least one head-injury that met diagnostic criteria for mTBI were classified as a TBI participant, and those never meeting criteria for TBI were classified as a MC participant.

Laboratory Genotyping Procedures

Participants were provided with a DNA collection kit and were instructed to swab the inside of their cheeks with cotton swabs to obtain saliva (buccal) samples that would be used for genotyping procedures. Participants' APOE genotype was determined using two single nucleotide polymorphism assays for the APOE coding regions (APOE112 and APOE158, rs429358 and APOE158, respectively). DNA was extracted from the buccal samples and quantified for polymerase chain reaction (PCR) amplification of APOE according to procedures

previously described in Saunders et al. (1993). Genotyping results for the overall sample were as follows: ϵ2/ϵ2 (n=0, 0%), ϵ2/ϵ3 (n=9, 9.1%), ϵ2/ϵ4 (n=1, 1.0%), ϵ3/ϵ3 (n=64, 64.6%), ϵ3/ϵ4 (n=23, 23.2%), and ϵ4/ϵ4 (n=2, 2.0%). Thus, 26 Veterans (26.3%) were ϵ4+ and 73 Veterans (73.7%) were ϵ4-. Veterans were blinded to their genotyping results.

Measures

The following comprehensive neuropsychological test battery was administered to all participants: California Verbal Learning Test-2nd Edition (CVLT-II; Delis et al., 2000); Design Fluency, Trail Making, and Verbal Fluency tests from the Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplan, & Kramer, 2001); Rey Complex Figure Test (RCFT; Meyers & Meyers, 1995); Block Design from the Wechsler Abbreviated Scale of Intelligence-Second Edition (WASI-II; Wechsler, 2011); Coding from the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV; Wechsler, 2008); Logical Memory and Visual Reproduction from the Wechsler Memory Scale-Fourth Edition (WMS-IV; Wechsler, 2009); and Wisconsin Card Sorting Test (WCST; Heaton, Chelune, Talley, Kay, & Curtiss, 1981). In addition, the Reading subtest of the Wide Range Achievement Test 4 (WRAT4; Wilkinson & Robertson, 2006) was administered to assess pre-morbid intellectual functioning. Finally, self-report measures were administered to assess current psychiatric functioning, including the Posttraumatic Stress Disorder (PTSD) Checklist – Military Version (PCL-M; Weathers, Huska, & Keane, 1991).

Approach to Data Analysis

Data Preparation and Reduction into Neurocognitive Composites: First, all neuropsychological variables of interest (24 in total) were converted from raw scores to

standardized scores (e.g., scaled scores, *T* scores, z scores) using manual-specific normative data. All standardized scores were then converted to standard scores (SS's), with a mean of 100 and *SD* of 15, so that all measures were on the same metric. Higher SS values reflect better performance. Next, to reduce the number of comparisons, domain-specific neurocognitive composites were derived using a theory-driven approach. In total, three composites were computed: (1) a memory composite, representing aspects of verbal and visual memory (8 indices); (2) a speed composite, representing tasks with a time demand (10 indices); and (3) an executive functioning composite, representing tasks related to cognitive set-shifting and problem solving (6 indices). The internal consistency of each composite was evaluated using Cronbach's alpha. Table 1 lists the variables comprising each domain-specific composite, as well as the associated Cronbach's alphas.

Deriving Impaired Scores: For the purpose of the present study, an impaired score was defined as any score falling >1.5 *SD*'s below the mean (<SS 78; Brooks, Iverson, Holdnack, & Feldman, 2008; Iverson & Brooks, 2011; Schretlen, Testa, Winicki, Pearlson, & Gordon, 2008). The total number of impaired scores across the test battery (possible range: 0-24) was computed for each participant. Veterans with two or more impaired scores were classified as "impaired," and Veterans with fewer than two impaired scores were classified as "not impaired." While there is no universal definition of cognitive impairment, consideration of the following factors resulted in using "two or more impaired scores" as the criterion to establish impairment groups: (1) the 1.5 *SD* threshold; (2) the number of tests administered; (3) base rates of impairment; and (4) the intellectual functioning of the sample (Binder, Iverson, & Brooks, 2009; Schretlen et al., 2008).

Statistical Analyses:

Participant Demographics: To determine whether there were any differences between mTBI and MC Veterans on basic demographic characteristics, independent-samples *t*-tests (for continuous data) and chi-square analyses (for categorical data) were used. The mTBI and MC groups were then further independently subdivided into APOE ϵ4 allele groups (ϵ4+ vs. ϵ4-) to determine whether there were group differences on demographic characteristics.

Neurocognitive Variables: Two-way analyses of variance (ANOVAs) were conducted to evaluate the effect of group (mTBI vs. MC) and ϵ4 status (ϵ4+ vs. ϵ4-) across the continuous neurocognitive variables (e.g., three composite scores and the total number of impaired scores). Additionally, chi-square analyses were used to examine the relation between ϵ4 status and cognitive impairment in both the mTBI and MC samples, respectively. Finally, given the high comorbidity between PTSD and mTBI, exploratory analyses of covariance (ANCOVAs)—controlling for PTSD—were conducted within the mTBI group to further evaluate the independent effect of ϵ4 status (ϵ4+ vs. ϵ4-) on the neurocognitive variables. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 24 (SPSS IBM, New York, NY).

RESULTS

Participant Demographics

Table 2 displays participant demographic characteristics for the overall sample, as well as for the mTBI and MC groups. The mTBI and MC groups were not significantly different across the majority of the demographic characteristics. However, the groups differed with respect to ethnicity (p=.036); specifically, a significantly greater number of Veterans in the MC group, relative to the mTBI group, identified as Caucasian.

As can be seen in Table 3, the mTBI group was, on average, 76.0 months post-injury (*SD*=50.64; *Med*=61 months; *Range*=20-267 months), and the average number of lifetime TBI's sustained by the group was 2.53 (*SD*=1.40; *Med*=2; *Range*=1-8), with the majority (75.5%) of the mTBI sample having a history of multiple (≥ 2) mTBI's. The mTBI and MC groups did not differ on APOE-ϵ4 status (Table 2) and, when stratified on APOE-ϵ4 status (Table 3), the mTBI groups do not significantly differ on any demographic or clinical variables measured as part of this study.

Neurocognitive Variables

Primary Analyses: Neurocognitive Composite Scores

Collapsed across the sample, results of a 2x2 ANOVA revealed a significant main effect of ϵ4 status on memory function, $F(1, 95) = 4.33$, $p = .040$, $\eta_p^2 = .044$, such that ϵ4+ Veterans ($M = 97.12$, $SD = 8.46$) performed more poorly on the memory composite than ϵ4- Veterans ($M = 101.93$, $SD = 10.97$). The main effect of group was not significant, ($F(1, 95) = 2.64$, $p = .107$; $\eta_p^2 = .027$), nor was the interaction between group and ϵ4 allele status ($F(1, 95) = 0.51$, $p = .475$; $\eta_p^2 = .005$). However, as can be gleaned by Figure 1, among Veterans with mTBI, the ϵ4+ group performed more poorly than the ϵ4- group ($F(1, 95) = 4.01$, $p = .048$, $\eta_p^2 = .041$); in contrast, among MCs, ϵ4 allele groups did not significantly differ on the memory composite ($F(1, 95) = 0.91$, $p = .343$, $\eta_p^2 = .009$).

Similarly, across the sample, a main effect was found for ϵ4 status on processing speed, $F(1, 95) = 8.08$, $p = .005$, $\eta_p^2 = .078$. Specifically, ϵ4+ Veterans ($M = 97.84$, $SD = 8.61$) performed more poorly than ϵ4- Veterans ($M = 102.89$, $SD = 7.79$). While the main effect of group approached significance (TBI < MC; $F(1, 95) = 3.39$, $p = .069$; $\eta_p^2 = .034$), there was not a

significant interaction between group and ϵ4 allele status ($F(1, 95) = 0.26$, $p = .609$; $\eta_p^2 = .003$). However, as indicated in Figure 2, among the mTBI participants, ϵ4+ Veterans showed worse performance on the speed composite relative to ϵ4- Veterans ($F(1, 95) = 5.78$, $p = .018$, $\eta_p^2 = .057$). Among MCs, APOE-ϵ4 status had no effect on the speed composite ($F(1, 95) = 2.65$, $p = .107$, $\eta_p^2 = .027$).

Finally, a trend was found for the main effect of ϵ4 status on executive function, $F(1, 95) = 2.96$, $p = .089$, $\eta_p^2 = .030$, such that ϵ4+ Veterans ($M = 97.81$, $SD = 10.37$) showed worse performance than ϵ4- Veterans ($M = 101.57$, $SD = 9.39$). However, the main effect of group was not significant, ($F(1, 95) = 0.16$, $p = .690$; $\eta_p^2 = .002$), and no interaction was found between group and ϵ4 allele status ($F(1, 95) = 0.06$, $p = .801$; $\eta_p^2 = .001$).

Primary Analyses: Number of Impaired Scores

Results of a 2x2 ANOVA revealed a significant main effect of ϵ4 status on the number of impaired scores across the neuropsychological battery, $F(1, 95) = 6.29$, $p = .014$, $\eta_p^2 = .062$. Specifically, collapsed across the sample, ϵ4+ Veterans ($M = 2.38$, $SD = 2.38$) displayed a greater number of impaired scores than ϵ4- Veterans ($M = 1.21$, $SD = 1.99$). The main effect of group was not significant, ($F(1, 95) = 2.83$, $p = .096$; $\eta_p^2 = .029$), nor was the interaction between group and ϵ4 allele status ($F(1, 95) = 0.80$, $p = .372$; $\eta_p^2 = .008$). Follow-up analyses (see Figure 3) revealed that among Veterans with mTBI, the ϵ4+ group demonstrated significantly more impaired scores than the ϵ4- group ($F(1, 95) = 5.95$, $p = .017$, $\eta_p^2 = .059$); however, among MCs, ϵ4 allele groups did not significantly differ on the number of impaired scores ($F(1, 95) = 1.27$, $p = .263$, $\eta_p^2 = .013$).

Chi-square analyses were independently conducted for the mTBI and MC groups. Among the mTBI sample, 22 of the 53 mTBI Veterans (41.5%) fell in the impaired group. A chi-square analysis revealed that a significantly higher proportion of mTBI ϵ4+ Veterans were impaired relative to mTBI ϵ4- Veterans, χ^2 (1, *N*=53) = 5.45, p = .020, φ = .32. Specifically, 69.2% (9 of 13) of ϵ4+ Veterans fell in the impaired group, compared with only 32.5% (13 of 40) of ϵ4- Veterans (see Figure 4). Among the MC sample, 14 of the 46 MC Veterans (30.4%) were classified as impaired. A chi-square analysis showed that there were no significant differences between ϵ4+ and ϵ4- Veterans on the proportion of participants who fell in the impaired group, χ^2 (1, *N*=46) = 2.12, p = .146, φ = .21); 46.2% (6 of 13) of ϵ4+ MC Veterans were impaired and 24.2% (8 of 33) of ϵ4- MC Veterans were impaired (see Figure 4).

Secondary Analyses:

Secondary analyses were conducted on the mTBI group only. ANCOVA's adjusting for PTSD symptoms (e.g., PCL-M total score) revealed a similar pattern of results as above. Specifically, when controlling for PTSD, the ϵ4+ and ϵ4- groups significantly differed on the memory (*F*(1, 48) = 4.96, p = .031, η_p^2 = .094) and speed (*F*(1, 48) = 6.32, p = .015, η_p^2 = .116) composites, with ϵ4+ participants generally showing poorer performance. However, as before, results revealed that there was no significant difference between ϵ4+ and ϵ4- allele groups for the executive functioning composite (*F*(1, 48) = 1.77, p = .190, η_p^2 = .036) when severity of PTSD symptoms was taken into account. Finally, after adjusting for PTSD symptom severity, mTBI ϵ4+ Veterans exhibited significantly more impaired scores across the cognitive battery when compared to the mTBI ϵ4- Veterans (*F*(1, 48) = 6.05, p = .018, η_p^2 = .112).

DISCUSSION

Previous studies examining the relationship between the APOE gene and neuropsychological outcome following TBI have overwhelmingly used disparate, heterogeneous samples, varying time intervals for post-injury assessment, and diverse neuropsychological tests and measurement strategies. Unfortunately, these methodological differences have resulted in conflicting and inconsistent findings regarding the effect of the ϵ4 allele on cognitive outcomes following TBI. Given the challenges associated with interpreting the existing literature, the present study utilized a narrowed approach to examine a specific cohort—Veterans with more common milder forms of head injury—who collectively were well beyond the initial phase of injury in order to better delineate associations between APOE-ϵ4 genotype and cognitive functioning.

Findings from the present study lend support to the notion that the ϵ4 genotype adversely influences cognitive performance in the context of mTBI. Specifically, we show that, among military Veterans with post-acute mTBI, ϵ4 carriers are at risk for (1) poorer mean neuropsychological performance in specific domains of functioning, including memory and processing speed, and (2) having a greater number of impaired scores across a comprehensive neuropsychological test battery. In contrast, in military controls, there were no significant differences in neuropsychological outcome (i.e., on mean neurocognitive composite scores and impaired scores) between ϵ4-carriers and non-carriers. These results suggest that the APOE ϵ4-genotype is particularly deleterious to neuropsychological functioning in the context of post-acute mTBI, but does not appear to have the same negative influence on neuropsychological functioning in the absence of neurotrauma. It is possible that possession of at least one copy of the ϵ4 allele may (1) result in increased risk for cognitive decline/impairment due to altered repair mechanisms following neurotrauma, or (2) exacerbate underlying mTBI-related cognitive

impairment. While more research in this area is needed, these results suggest that the combination of TBI and ϵ4-positivity increases the likelihood of cognitive dysfunction, even in participants with milder forms of head injury.

Despite methodological differences between the present study and previous studies examining the APOE gene and cognitive functioning following TBI, our data are relatively consistent with other work (Ariza et al., 2006; Crawford et al., 2002; Eramudugolla et al., 2014; Lawrence et al., 2015; Liberman et al., 2002; Merritt et al., 2017; Sundström et al., 2004). In particular, our finding that ϵ4+ mTBI Veterans performed more poorly on memory functioning compared to ϵ4- mTBI Veterans dovetails with the results of Ariza et al. (2006), Crawford et al. (2002), and Eramudugolla et al. (2014) who also reported reduced memory performance in TBI ϵ4+ participants. Furthermore, as shown in the present study, differences in speed-related tasks between ϵ4+ and ϵ4- TBI participants were also reported by Ariza et al. (2006) and Liberman et al. (2002). In contrast, our findings diverge from other studies that have reported no significant differences on cognitive performance between ϵ4+ and ϵ4- TBI participants (Chamelian et al., 2004; Hodgkinson et al., 2009; Millar et al., 2003; Padgett, Summers, Vickers, et al., 2016; Ponsford et al., 2007; Pruthi et al., 2010; Shadli et al., 2011). As mentioned previously, these discrepancies may be largely attributable to contrasting sample characteristics, sample blending (i.e., examining mild, moderate, and/or severe TBI together), and other methodological differences.

We also showed that, across the entire neuropsychological battery, the mTBI ϵ4+ group displayed significantly more impaired scores relative to the mTBI ϵ4- group. Furthermore, after independently dichotomizing the mTBI and MC groups into “impaired” and “not impaired” groups (using a cutoff of ≥ 2 impaired scores falling 1.5 *SD*’s below the mean to establish

impairment), a significantly greater proportion of mTBI ϵ4+ Veterans were impaired relative to mTBI ϵ4- Veterans. These findings are consistent with prior work (Merritt et al., 2017), which represents the only other known study to have examined the APOE gene and neurocognitive impairment post-TBI. Similar to our results discussed above, no significant differences were found between ϵ4+ and ϵ4- military control Veterans on the number of impaired test scores, providing further evidence to suggest that ϵ4 genotype specifically exacerbates neurocognitive impairment in the presence of CNS compromise. Importantly, our findings held after adjusting for PTSD symptoms, indicating that the presence of an ϵ4 allele negatively influences cognitive functioning following mTBI even when accounting for PTSD severity. This is especially relevant given previous work showing that the ϵ4 allele is associated with elevated PTSD symptoms in Veterans with a history of mild to moderate TBI (Merritt et al., under review).

At present, the precise mechanisms responsible for the negative effects of the ϵ4 allele on cognitive functioning following mTBI are not well established. However, a large body of research supports the notion that, compared to the ϵ2 and ϵ3 alleles, the ϵ4 allele of the APOE gene possesses a number of detrimental properties that may hinder recovery following neurological injury (for a review, see Mahley et al., 2006; Small et al. 2004; Wisdom, Callahan, & Hawkins, 2011). For example, ϵ4 genotype status is associated with increased cerebral edema and brain inflammation (Laskowitz et al., 1998; Lynch et al., 2002), diminished neural repair properties/reduced neurite outgrowth (Bellosta et al., 1995; Nathan et al., 1994), decreased lipid transport efficiency (Kay et al., 2003), and greater accumulation of Aβ (Nicoll et al., 1995; Ji et al., 2002; Holtzman et al., 2000; Mahley et al., 2006). Furthermore, there is recent evidence to suggest that the ϵ4 allele markedly exacerbates tau-mediated neurodegeneration (Shi et al.,

2017). Thus, it is possible that the APOE-ϵ4 genotype contributes to poorer, or slower, brain recovery that in turn negatively influences cognitive performance following TBI.

Findings of the present study also showed that memory function and processing speed are most susceptible to the negative effects of the ϵ4 allele, suggesting that perhaps certain brain regions are particularly affected by the ϵ4 genotype. Indeed, recent imaging work in both younger and older adult populations has shown that ϵ4-carriers have reduced hippocampal volumes relative to non-carriers (Alexopoulos et al., 2011; Liu et al., 2016; O'Dwyer et al., 2012). Such work may help to explain the differences in memory performance observed between ϵ4+ and ϵ4- mTBI Veterans in our study.

Our study has several strengths, including a well-characterized sample of younger Veterans with history of mild head injury, a focus on a well-defined time period post-injury (post-acute TBI), use of multiple methods for examining neuropsychological performance, and a rare emphasis on genetic-cognition associations with mTBI history. However, there are important limitations of the present study that should be considered. First, given the makeup of our sample, findings are likely not generalizable to non-military samples, females, adolescent and older adult samples, patients with more severe TBI, and patients with a history of only a single head injury. Results may also be less pertinent to those who are in the acute to subacute phase of injury. Moreover, our data show a low number of participants who were homozygous for the ϵ4 allele (n=2), and therefore a dose effect was unable to be evaluated. Future studies with larger samples will be in a better position to examine these associations in a dose-response fashion.

In conclusion, while a number of previous studies have examined the APOE gene within the context of brain injury, this is the first study, to our knowledge, to examine the relationship in

the post-acute phase of injury, examining only Veterans with a history of mTBI and evaluating multiple aspects of neuropsychological functioning. Our findings suggest that the APOE-ϵ4 genotype has a negative influence on aspects of cognition in the context of post-acute mTBI, but does not appear to have the same negative effects on cognition in the absence of neurotrauma. The present study advances our current understanding of these relationships by showing that APOE-ε4 genotype is associated with cognitive impairment even well beyond the acute phase of head injury and within the context of milder forms of TBI. Future work is necessary to elucidate the underlying brain-based mechanisms of ε4 allelic effects—and other genes beyond APOE—on cognitive and clinical outcomes following TBI in order to enhance our understanding of the specific factors that influence recovery following brain injury.

Table 1. Variables comprising each domain-specific composite.

Memory Composite	Speed Composite	Executive Functioning Composite
CVLT Trials 1-5 Total Recall	WASI-II Block Design	WCST Perseverative Errors
CVLT Short Delay Free Recall	WAIS-IV Coding	WCST Total Errors
CVLT Long Delay Free Recall	D-KEFS Trail Making Visual Scanning	D-KEFS Verbal Fluency Switching
WMS-IV Logical Memory I	D-KEFS Trail Making Number Sequencing	D-KEFS Verbal Fluency Switching Accuracy
WMS-IV Logical Memory II	D-KEFS Trail Making Letter Sequencing	D-KEFS Trail Making Switching
WMS-IV Visual Reproduction I	D-KEFS Trail Making Motor Speed	D-KEFS Design Fluency Switching
WMS-IV Visual Reproduction II	D-KEFS Design Fluency Empty	
Rey-O Delay	D-KEFS Design Fluency Filled	
	D-KEFS Verbal Fluency Letter	
	D-KEFS Verbal Fluency Category	
Cronbach's α = .843	**Cronbach's α = .850**	**Cronbach's α = .760**

Table 2. Demographic characteristics.

Variables	Overall Sample (n=99)		mTBI Group (n=53)		MC Group (n=46)		
	M	**SD**	**M**	**SD**	**M**	**SD**	***p*[a]**
Age	32.43	6.77	32.40	6.37	32.48	7.27	.952
Education (Years)	14.58	1.77	14.28	1.61	14.91	1.91	.078
WRAT4 Reading SS	102.83	10.74	101.75	11.86	104.04	9.28	.294
PCL-M Total Score	34.08	18.31	43.53*	17.98	23.61	12.27	**<.001**
	N	**%**	**N**	**%**	**N**	**%**	***p*[b]**
Sex							
Male	77	77.8	45	84.9	32	69.6	.067
Female	22	22.2	8	15.1	14	30.4	
Ethnicity							
Caucasian	58	58.6	24	45.3	34	73.9	
Hispanic	24	24.2	18	34.0	6	13.0	**.036**
African American	6	6.1	4	7.5	2	4.3	
Asian/Pacific Islander	8	8.1	6	11.3	2	4.3	
Other	3	3.0	1	1.9	2	4.3	
Branch of Service							
Air Force	9	9.1	5	9.4	4	8.7	
Army	22	22.2	13	24.5	9	19.6	.556
Marines	37	37.4	22	41.5	15	32.6	
Navy	28	28.3	12	22.6	16	34.8	
Missing	3	3.0	1	1.9	2	4.3	
Married/Cohabitating							
Yes	39	39.4	20	37.7	19	41.3	.834
No	58	58.6	31	58.5	27	58.7	
Missing	2	2.0	2	3.8	0	0	
Currently Employed							
Yes	47	47.5	21	39.6	26	56.5	.131
No	50	50.5	30	56.6	20	43.5	
Missing	2	2.0	2	3.6	0	0	
APOE ϵ4 allele Status							
ϵ4 Present (ϵ4+)	26	26.3	13	24.5	13	28.3	.674
ϵ4 Absent (ϵ4-)	73	73.7	40	75.5	33	71.7	

Abbreviations: mTBI = mild traumatic brain injury; MC = military control; WRAT4 = Wide Range Achievement Test 4; SS = standard score; PCL-M = Posttraumatic Stress Disorder (PTSD) Checklist – Military Version. [a]Independent samples *t*-tests were conducted to evaluate groups on age, education, WRAT4 Reading performance, and PCL-M total score. [b]Chi-square analyses were conducted to evaluate groups on sex, ethnicity, branch of service, marital status, employment status, and APOE ϵ4 allele status.

*The PCL-M total score for the mTBI participants is based on N=51, as two Veterans were missing the PCL-M total score.

Table 3. Mild TBI participant demographic and injury-related characteristics by ε4 allele group (N=53).

Variables	ε4 allele Present (ε4+) (n=13)		ε4 allele Absent (ε4-) (n=40)		
	M	**SD**	**M**	**SD**	***p*[a]**
Age	32.54	6.85	32.35	6.30	.927
Education (Years)	13.85	1.46	14.43	1.65	.264
WRAT4 Reading SS	101.00	11.11	102.00	12.23	.795
Total # of TBI's	2.08	1.04	2.68	1.47	.182
Months Since Most Recent TBI	56.00	35.91	82.48	53.35	.102
	N	**%**	**N**	**%**	***p*[b]**
Sex					
Male	11	84.6	34	85.0	.973
Female	2	15.4	6	15.0	
Ethnicity					
Caucasian	3	23.1	21	52.5	.070
Hispanic	6	46.2	12	30.0	
African American	3	23.1	1	2.5	
Asian/Pacific Islander	1	7.7	5	12.5	
Other	0	0	1	2.5	
TBI Due to Blast Exposure					
Yes	5	38.5	14	35.0	.821
No	8	61.5	26	65.0	
LOC (most sig. TBI)*					
Yes	6	46.2	24	60.0	.382
No	7	53.8	16	40.0	
AOC (most sig. TBI)*					
Yes	7	53.8	16	40.0	.382
No	6	46.2	24	60.0	
PTA (most sig. TBI)*					
Yes	7	53.8	21	52.5	.933
No	6	46.2	19	57.5	

Abbreviations: WRAT4 = Wide Range Achievement Test 4; SS = standard score; TBI = traumatic brain injury; LOC = loss of consciousness; AOC = alteration of consciousness; PTA = post-traumatic amnesia. [a]Independent samples *t*-tests were conducted to evaluate groups on age, education, WRAT4 Reading performance, total number of TBI's sustained, and time from most recent TBI to testing. [b]Chi-square analyses were conducted to evaluate groups on sex, ethnicity, presence of blast exposure, and presence of LOC, AOC, and PTA.

*For Veterans with a history of >1 mTBI, the presence of LOC, AOC, and PTA was determined based on their "most significant" TBI experienced.

Figure Captions

Figure 1. Memory composite scores (in standard score units; M=100, SD=15) across military controls and mTBI Veterans by ε4 allele group. Mean scores (± standard errors) are displayed. *p<.05.

Figure 2. Processing speed composite scores (in standard score units; M=100, SD=15) across military controls and mTBI Veterans by ε4 allele group. Mean scores (± standard errors) are displayed. *p<.05.

Figure 3. Total number of impaired scores across military controls and mTBI Veterans by ε4 allele group. Mean scores (± standard errors) are displayed. *p<.05.

Figure 4. Proportion of Veterans classified as "impaired" across military controls and mTBI Veterans by ε4 allele group. *p<.05.

Figure 1.

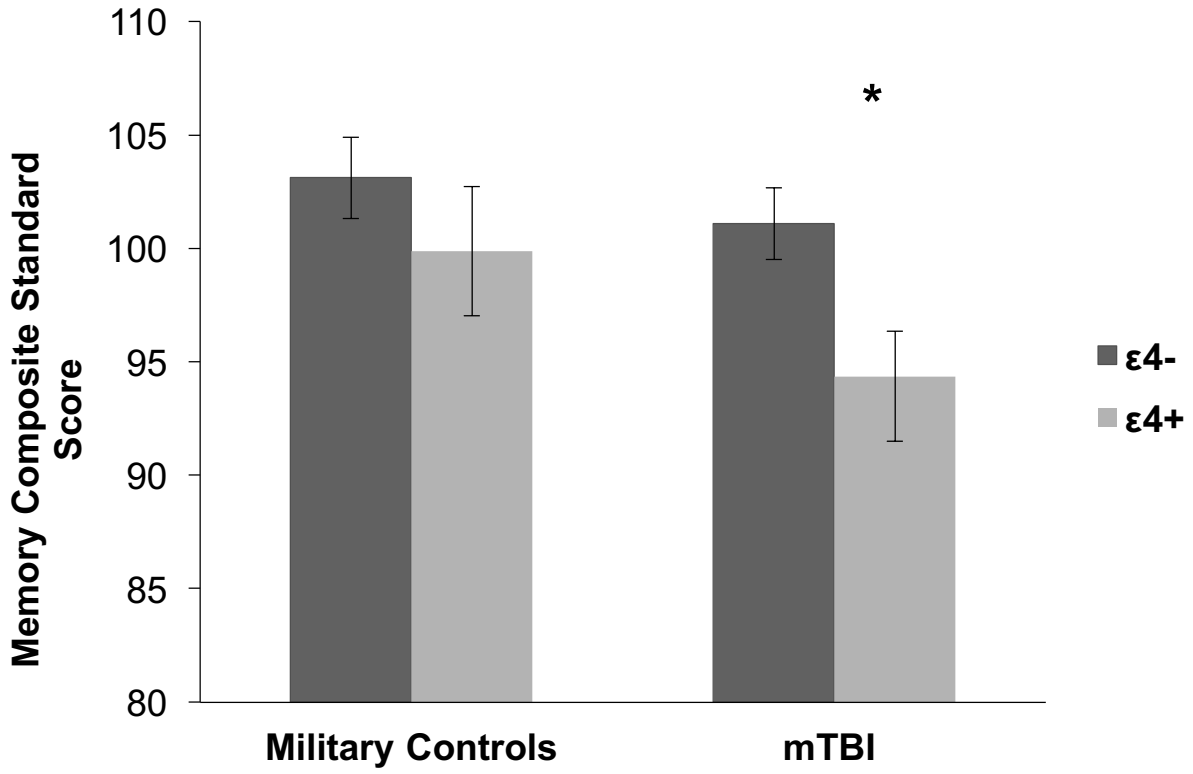

110
105
100
95
90
85
80
Memory Composite Standard Score
*
Military Controls
mTBI
ε4-
ε4+

Figure 2.

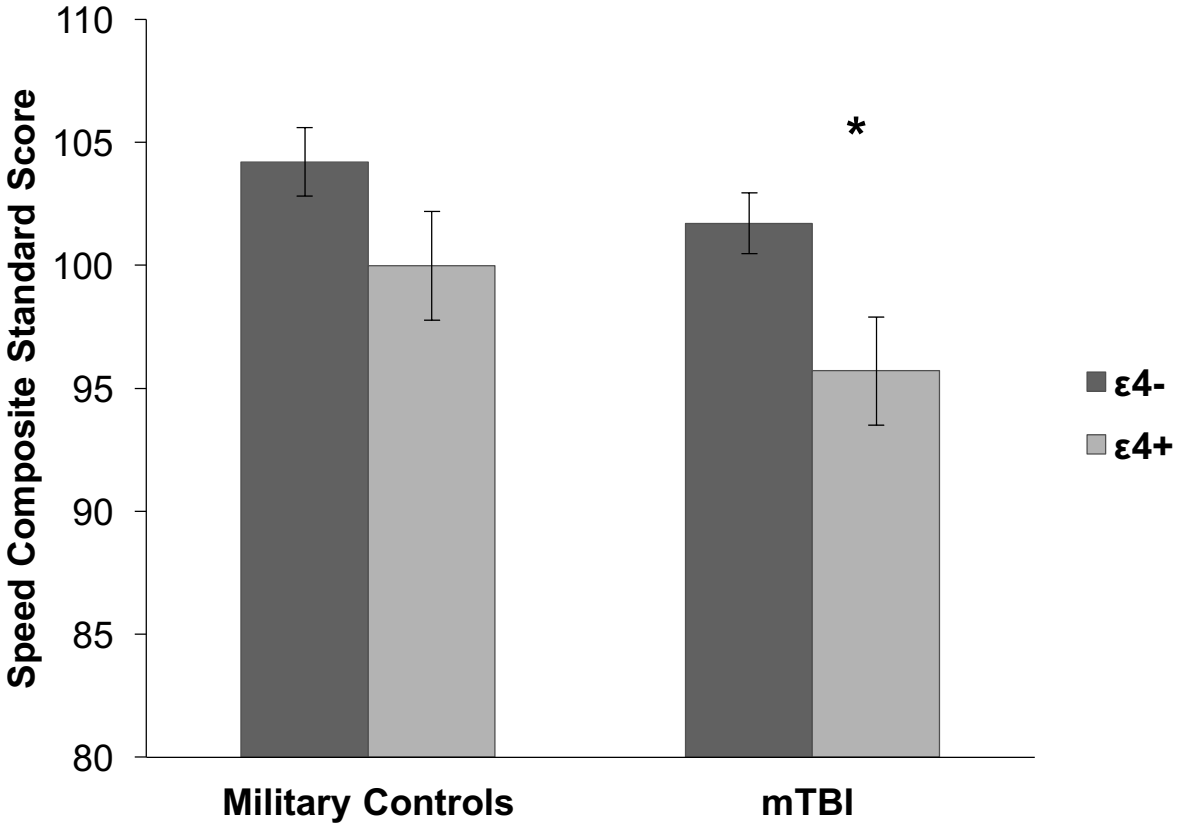

110
105
100
95
90
85
80
Speed Composite Standard Score
*
Military Controls
mTBI
ε4-
ε4+

Figure 3.

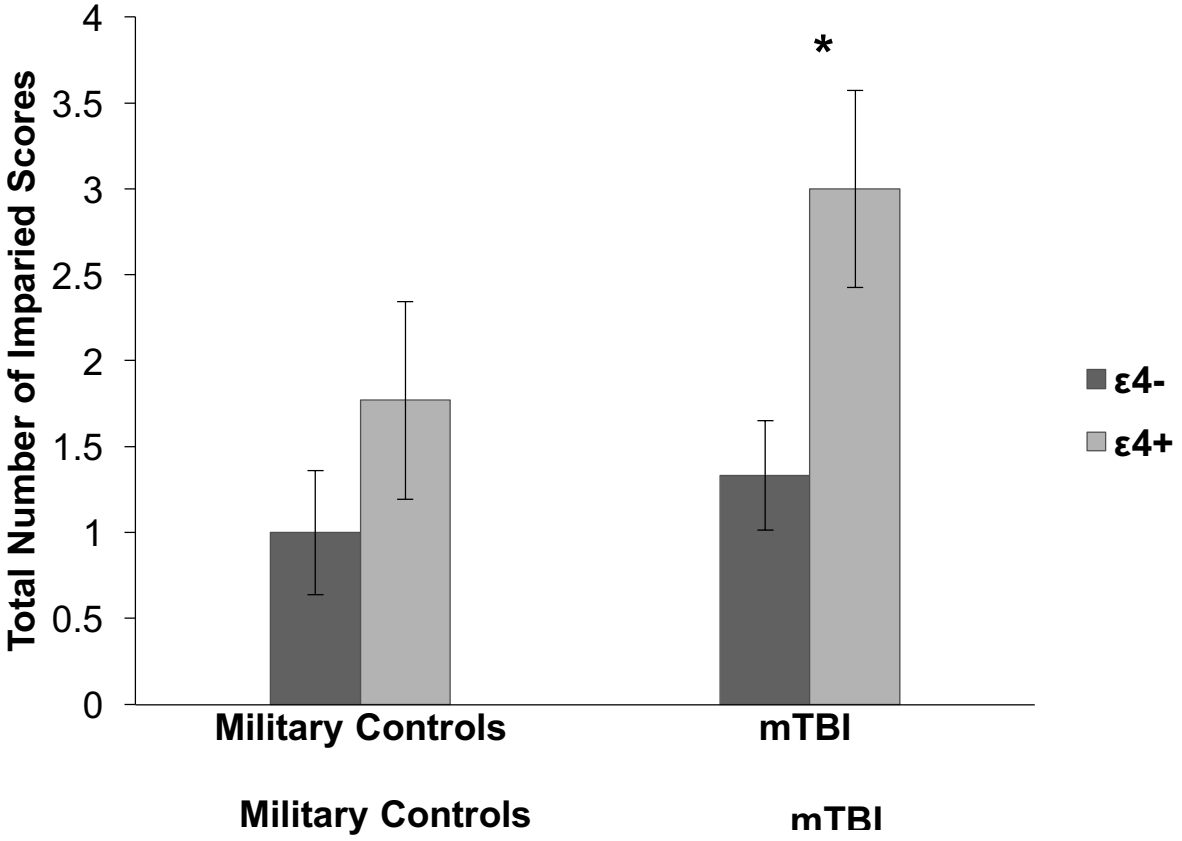
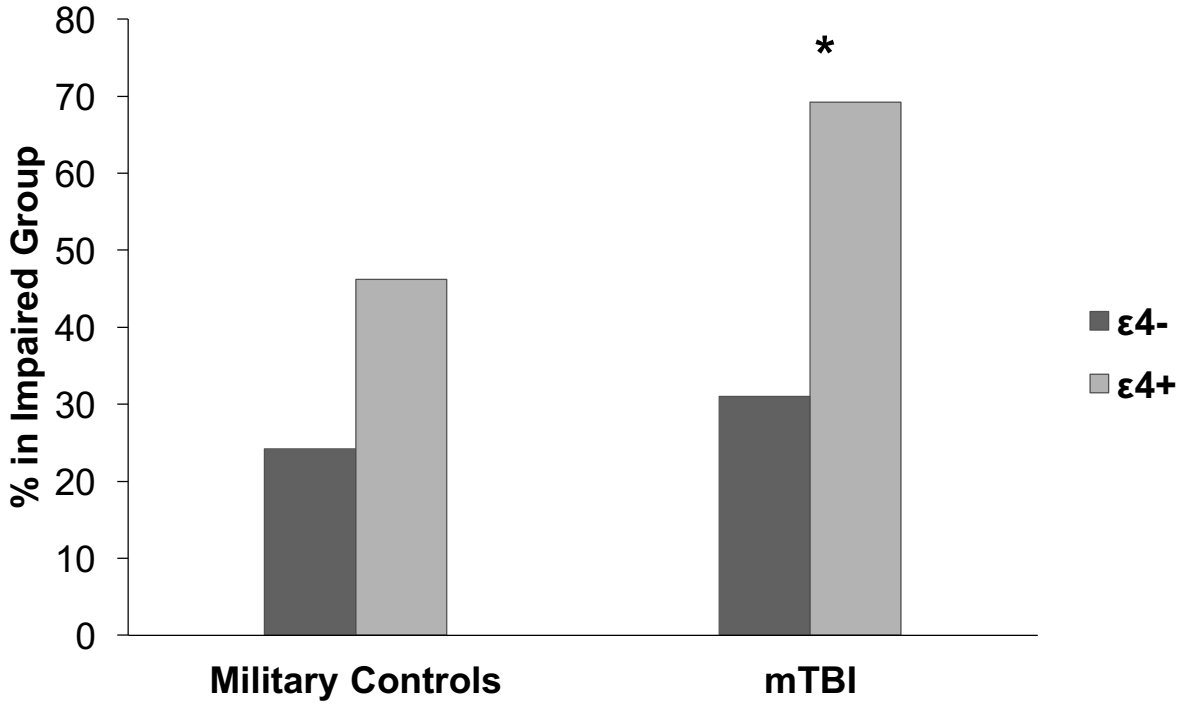

Total Number of Imparied Scores
4
3.5
3
2.5
2
1.5
1
0.5
0
*
ε4-
ε4+
Military Controls
mTBI
Military Controls
mTBI

Figure 4.


80
70
60
50
40
30
20
10
0
% in Impaired Group
*
Military Controls
mTBI
ε4-
ε4+

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Full Abstracts Presented in 2017:

Neural Correlates of Response Inhibition in Mild-Moderate Traumatic Brain Injury: An fMRI Study

Kelsey A. Holiday, Lisa T. Eyler, Russell T. Kim, Scott Sorg, Alexandra L. Clark, Lisa Delano-Wood, Dawn M. Schiehser

Introduction: Response inhibition has been theorized to be impaired in individuals with Traumatic Brain Injury (TBI), yet many studies fail to detect group differences in performance on response inhibition tasks between individuals with TBI and healthy controls. It is presumed that cognitive compensation secondary to neuronal injury may contribute to this observed behavior. However, this has yet to be experimentally tested in mild-moderate TBI (mmTBI). Thus, the objective of our study was to examine the neural correlates of response inhibition in mmTBI.

Methods: Sixty-nine Veterans with mmTBI and 41 Veteran Controls (VC) participated in a go/no-go task while undergoing event-related functional magnetic resonance imaging (fMRI). After field inhomogeneity and motion correction, spatial smoothing, and registration to standard atlas space, changes from baseline in the blood oxygen-level dependent signal during the no-go trials were calculated at each voxel and maps were compared between groups with independent sample t-tests. Task performance was measured by d-prime, or discriminability accuracy, calculated by subtracting the z-score for the false alarm rate from the z-score of the hit rate.

Results: The mmTBI participants did not significantly differ from VC on go/no-go task performance (d prime). During the inhibition trials ("no-go"), VC exhibited greater deactivation in the right anterior cingulate cortex (rACC) compared to mmTBI participants. Within the mmTBI group, worse performance was associated with greater rACC activation.

Discussion: Compared to controls, mmTBI Veterans exhibited difficulty deactivating the rACC, and this impaired deactivation was associated with worse response inhibition. These findings suggest that mmTBI disrupts brain function necessary for adequate response inhibition and underscore the importance of the rACC in response inhibition.

Brain Function and Task Performance Predict Self-Reported Disinhibition and Executive Function in Veterans with Mild-Moderate Traumatic Brain Injury

Russell T. Kim, Scott F. Sorg, Kelsey A. Holiday, Lisa Delano-Wood, M.J. Meloy, Alexandra L. Clark, My Tran, Lyzette E. Locano, Lisa T. Eyler, Dawn M. Schiehser

Objective: Veterans often report neurobehavioral and cognitive changes following mild-moderate traumatic brain injury (mmTBI); however it is unclear whether self-reported complaints including disinhibition and executive dysfunction (EDF) are related to objective cognitive performance and brain function, especially in the context of PTSD. We therefore examined whether objective cognitive performance, brain activation, and/or PTSD symptoms best predicted self-reported disinhibition and EDF in mmTBI Veterans.

Participants and Methods: 56 Veterans with mmTBI and 35 Veteran controls (VC) completed the Frontal Systems Behavior Scale (FrSBe), a measure of subjective disinhibition and EDF, and PTSD Checklist-Military. Participants also completed the Go/NoGo task, a cognitive inhibition task, while undergoing fMRI.

Results: Groups did not differ on Go/NoGo performance (d') or BOLD signal change, but the mmTBI group reported higher FrSBe scores and PTSD (p's<0.05) compared to VCs. In the mmTBI group, subjective disinhibition and EDF were related to worse d' and PTSD (p's<.02). Greater levels of subjective disinhibition were associated with increased signal change in the bilateral rostral anterior cingulate (AC; p's<.01), while EDF was associated with increased signal change in the bilateral caudal AC, left rostral AC, and precentral gyrus (p's<0.03). In regression analyses, increased signal change in the right rostral AC, d', and PTSD all predicted subjective disinhibition; EDF was predicted by PTSD and increased signal change in the left precentral gyrus (p's<.04).

Conclusions: This study represents one of the first to empirically link subjective disinhibition and executive dysfunction with objective cognition and brain function in mmTBI. Subjective disinhibition and EDF appear to be dissociated by differing neural mechanisms and possible subsequent decrements in task performance. Findings underscore the importance of detailed measurement and highlight the potential utility of neuroimaging to elucidate neurobehavioral changes in mmTBI.

Elevated Intra-individual Variability on Tests of Executive Functions in Veterans with Mild Traumatic Brain Injury

Scott F. Sorg, Alexandra L. Clark, Madeleine L. Werhane, Russell T. Kim, Dawn M. Schiehser, Mark W. Bondi, Lisa Delano-Wood

Objective: Although cognitive disruption in mild TBI (mTBI) may be non-uniform, few studies have explored within-subject variations across cognitive tests. Given that efforts investigating mTBI outcomes have largely focused on comparison of mean levels of performance which may obscure group differences, we examined whether intra-individual variability (IIV) across tests of executive functions (EF) is increased in mTBI relative to controls in a sample of military Veterans. We also explored relationships among EF-IIV, PTSD symptoms and white matter integrity.

Participants and Methods: 78 Veterans (mTBI=44, Military Controls [MCs]=34) completed PTSD ratings, diffusion tensor imaging (DTI), and cognitive testing with optimal effort testing. EF-IIV was calculated as the standard deviation across 5 tests of EF (DKEFS Trails Letter/ Number Switching, Verbal Fluency Switching, Letter Fluency and Design Fluency Switching; and WCST Perseverative Responses). Fractional anisotropy (FA) was extracted from selected white matter ROIs.

Results: Compared to MCs, the mTBI group had higher EF-IIV (p.05). EF-IIV negatively correlated with FA in multiple frontal white matter regions (r's=-.28-39, p's.05).

Conclusions: Results show that Veterans with mTBI have greater variability across EF tests compared to MCs above and beyond what may be attributed to mean group differences on those measures or psychiatric symptoms. Findings suggest that, in contrast to conventional analyses that average scores across tests, discrepancy analyses may more sensitively capture cognitive impairment and demonstrate tighter associations with imaging indices in Veterans with head injury histories.

Repetitive Mild Traumatic Brain Injury Moderates the Association Between Age and Cerebral Blood Flow of Medial Temporal Lobe Structures.

Alexandra L. Clark, Katherine J. Bangen, Scott F. Sorg, Madeleine L. Werhane, Dawn M. Schiehser, Mark W. Bondi, Lisa Delano-Wood

Objective: Traumatic brain injury (TBI)—even when mild in severity— is a risk factor for poor long-term outcomes and neurodegenerative changes, and vascular pathology may play an important role. Although cerebral blood flow (CBF) alterations have been observed in both TBI and aging, little is known about how history of repetitive TBI may influence CBF in regions that are also vulnerable to aging. We therefore sought to clarify the influence of repetitive TBI and age on CBF, as well as associations between CBF and cognition.

Participants and Methods: 37 Veterans with history of TBI (mean age = 35) underwent neuroimaging, completed psychiatric symptom inventories, and were administered a comprehensive neuropsychological battery. Participants were divided into two groups: (1) those with history of 1 or 2 TBIs (n=19) and (2) those who have sustained 3+ TBIs (n=18). Resting CBF was measured using multiphase pseudocontinuous arterial spin labeling and averaged across FreeSurfer-derived parcellations of the medial temporal lobe (MTL).

Results: Regression analyses, adjusting for symptoms of posttraumatic-stress disorder (PTSD), revealed a significant Group x Age interaction for resting CBF in the right fusiform (p=.03) and parahippocampal gyri (p=.008), and a trend was observed in bilateral hippocampi (p's=.08). Examination of simple main effects revealed that increased age was significantly associated with decreased CBF in those with 3+ TBIs, but not in those with fewer injuries. Across the sample, regression analyses adjusting for PTSD revealed that reduced CBF was associated with poorer verbal memory performance (p's< .05).

Conclusions: Lifetime history of repetitive TBIs exacerbates age-related CBF reductions of the MTL. Given this region is known to be susceptible to neurodegenerative diseases, our findings suggest that CBF alterations following multiple TBIs may contribute to the deleterious pathological processes that give rise to poor cognitive outcomes.

Brain Derived Neurotropic Factor (BDNF) Val66Met Moderates the Association Between PTSD and Cortical Thickness in Veterans with History of Traumatic Brain Injury.

Nicole D. Evangelista, Alexandra L. Clark, Katherine J. Bangen, Scott F. Sorg, Madeleine L. Werhane, Dawn M. Schiehser, Lisa Delano-Wood

Objective: Post-traumatic stress disorder (PTSD) has been linked to cortical thinning of frontal and temporal regions in Veterans with history of traumatic brain injury (TBI). However, it remains unclear how brain-derived neurotrophic factor (BDNF)—a protein that plays a role in neuronal growth, maturation and maintenance—may potentially influence the effects of PTSD on the brain. We therefore sought to clarify the associations between BDNF genotype, PTSD, and cortical thickness in Veterans with history of mild TBI (mTBI).

Participants and Methods: 59 Veterans with history of mTBI underwent BDNF genotyping and were divided into (1) Met+ carrier (n=29) and (2) Met- carrier (n=30) subgroups. Participants also completed psychiatric symptom questionnaires (PCL-M) and structural MR imaging. Cortical thickness values were derived from parcellated regions of interest (ROIs) using FreeSurfer.

Results: Analysis of covariance controlling for age, revealed a significant PTSD x Genotype interaction for cortical thickness in the cuneus, precuneus, and the rostral anterior cingulate (p's $< .05$).

Conclusions: Results show that BDNF genotype differentially affects the relationship between PTSD and cortical thickness in Veterans with history of mTBI. Specifically, mTBI Met+ carriers—who may have lower levels of this critical neurotrophin available in the central nervous system— appear to be especially vulnerable to the negative effects of PTSD on cortical thickness of several brain regions. These findings suggest that BDNF genotype plays an important role in modulating brain structure in the context of comorbid TBI and PTSD. Future studies should further evaluate the epigenetic effects of BDNF on recovery and treatment outcomes in Veterans with comorbid TBI and PTSD.

Lower Nucleus Accumbens Volume is Associated with Reduced Reward-Based Decision Making in Veterans with History of Mild Traumatic Brain Injury.

Samreen Haque, Alexandra L. Clark, Nicole D. Evangelista, Madeleine L. Werhane, Scott F. Sorg, Dawn M. Schiehser, Lisa Delano-Wood

Mild traumatic brain injury (mTBI) has been associated with diffuse neuronal loss, and recent research suggests that neurodegeneration in subcortical regions may play a fundamental role in commonly observed negative behavioral outcomes post-injury. The nucleus accumbens—a structure critical for motivation, reward, and higher-order decision-making— may be especially vulnerable to TBI-induced damage. Therefore, in a well-characterized sample of Veterans, we sought to (1) clarify whether volumetric differences can be observed following mTBI and (2) explore associated brain-behavior relationships. Our sample included 73 Veterans with (n = 38) and without history of mild TBI (n = 35) who underwent structural magnetic resonance imaging

(MRI) and were administered a computerized version of the Iowa Gambling Task (IGT). FMRIB's Software was used to obtain whole brain and nucleus accumbens volume estimates. Analyses of covariance controlling for intracranial volume (ICV) and post-traumatic stress disorder (PTSD) symptoms were used to determine whether there were group differences in lateralized nucleus accumbens volumes. Partial correlations were used to explore brain-behavior associations across the groups. Results revealed that independent of ICV, the mTBI group displayed significantly lower volumes of the left nucleus accumbens volumes relative to Veterans without history of mTBI ($p = .04$). When PTSD was taken into account, this relationship became a trend ($p = .08$). Additionally, across both groups, partial correlations demonstrated that independent of ICV and PTSD, lower left nucleus accumbens volume was significantly associated with poorer performance on a test of reward-based decision making (IGT Total Raw Score, $r = 0.25$, $p = 0.04$). Our results show that the nucleus accumbens may be vulnerable to neurodegenerative changes following mild neurotrauma. Importantly, gray matter volumetric changes in this region may contribute to impaired decision making in the aftermath of TBI. Findings suggest a neuroanatomical basis or mechanism for reduced executive function which is frequently observed in individuals who have sustained even mild levels of brain trauma. Future studies integrating other biomarkers of neurodegeneration and inflammation may assist in clarifying the mechanisms underlying volumetric changes post-injury.

Apolipoproptein E-e4 Genotype and Pulse Pressure Interact to Affect Cortical Thickness in Brain Regions Vulnerable to Alzheimer's Disease in Veterans with Mild Traumatic Brain Injury.

Kristina M. Lapira, Madeleine L. Werhane, Alexandra L. Clark, Nicole D. Evangelista, Scott F. Sorg, Dawn M. Schiehser, Mark W. Bondi, Lisa Delano-Wood

Objective: Vascular, genetic, and environmental (e.g., mild traumatic brain injury [mTBI]) factors have all been shown to increase risk for development of Alzheimer's disease (AD) in late life. Although there has been extensive research linking the APOE-ε4 allele and risk for AD risk in mTBI samples, it remains unclear how vascular risk might interact with the presence of the APOE-e4 allele in the expression of brain changes in participants with history of head-injury. Thus, the present study explored the independent and interactive effects of elevated vascular risk and APOE-ε4 positivity on cortical thinning in brain regions vulnerable to AD pathology in Veterans with history of mTBI.

Participants and Methods: Participants included 48 Veterans (mean age: 32; range: 23-46) with history of mTBI who underwent magnetic resonance imaging (MRI), APOE genotyping, and blood pressure (BP) assessment. Pulse pressure (PP), an index of arterial stiffening, was derived from blood pressure values. FreeSurfer was used to generate cortical thickness values for brain regions of interest (ROIs).

Results: Multiple linear regression, adjusting for age and PTSD, showed that APOE-ε4 positivity modified the relationship between PP and cortical thickness in medial orbitofrontal ($p = .023$), precentral ($p = .091$), supramarginal ($p = .082$), and precuneus (.045) regions. For each of these ROIs, lower PP was associated with greater cortical thinning in ε4-positive participants. Conversely, higher PP was associated with cortical thinning in these same regions in non-ε4 carriers.

Conclusions: Results revealed that, even within a relatively young sample of participants with mild head trauma histories, vascular and genetic risk interact to affect cortical thickness in AD vulnerable regions. Specifically, mTBI participants with lower PP and who possessed at least one copy of the ε4 allele showed cortical thinning across several regions known to be predilection sites for AD pathology. However, contrary to our hypothesis, higher PP appeared to be protective against cortical thinning in APOE-ε4 carriers, but not non-carriers. While additional research is needed to clarify these findings, they add to a growing literature that highlights the complexity of vascular and genetic contributions to AD pathology following neurotrauma.

Global and Regional Thalamic Morphometry is Associated with Fatigue in Veterans with History of Mild Traumatic Brain Injury

Alexandra L. Clark, M.S., Dawn M. Schiehser, Ph.D., Scott F. Sorg, Ph.D., Katherine J. Bangen, Ph.D., Madeline Werhane, B.A., Kelsey Holiday, B.A., & Lisa Delano-Wood, Ph.D.

Objective**:** Fatigue is a common, chronic, and oftentimes disabling symptom following traumatic brain injury (TBI). Although the neural underpinnings of fatigue remain poorly understood, emerging evidence suggests the thalamus may play a pivotal role in the manifestation of fatigue symptoms. The thalamus—a central relay station with vast cortical connections responsible for regulation of consciousness, sleep, and sensory interpretation—has been demonstrated to be especially susceptible to primary and secondary damage following TBI. Therefore, the current study sought to investigate whether fatigue symptoms are associated with global and regional thalamic morphometry (i.e., volume and shape changes) post-TBI.

Methods**:** 63 Veterans (age range: 21-50; mean = 32) with history of mild TBI underwent structural magnetic resonance scanning (MRI) and completed questionnaires related to fatigue and psychiatric symptomatology. FMRIB's Software (FSL) was used to obtain whole brain and thalamic volume estimates, as well as to perform lateralized vertex-wise shape analyses. Linear regression was used to model associations between thalamic volume or shape correcting for age, sex, intracranial volume, and PTSD symptoms.

Results**:** Regression analyses revealed that elevated fatigue ratings were a significant predictor of reduced right thalamic volume ($p = .02$), while there was a trend for left thalamic volume ($p = .052$). Vertex-based shape analyses revealed that fatigue ratings were significantly associated with regional atrophy in dorso-medial and anterior regions of the right thalamus ($p < .05$); a similar trend was observed in anterior aspects of the left thalamus ($p = .07$).

Conclusions**:** Our results demonstrate that self-reported fatigue–a frequently reported and often enduring symptom in Veterans who have sustained a TBI event—is associated with global and local morphometry (volume and shape) of the thalamus in Veterans with history of mild neurotrauma. Specifically, our findings suggest that regional changes in the right dorsomedial and anterior region of the thalamus may be implicated in fatigue. Importantly, projections to and from these areas extend to frontal, temporal, and sensory areas that play a fundamental role in

self-valuation, awareness and higher-order cognitive functions. Future studies should investigate components of fatigue (e.g., physical and cognitive) and integrate multiple MR methods in order to expand upon our understanding of the underlying neural and functional consequences of TBI.

Apolipoprotein E (APOE) e4 Genotype is Associated with Increased Psychiatric Distress in Veterans with a History of Mild-to-Moderate Traumatic Brian Injury

Victoria C. Merritt, Alexandra L. Clark, Scott F. Sorg, Nicole D. Evangelista, Mark W. Bondi, Dawn M. Schiehser, Lisa Delano-Wood

Objective: Since few studies have examined the relationship between the APOE gene and long-term clinical outcomes following traumatic brain injury (TBI), we aimed to determine whether the e4 allele of the APOE gene influences neuropsychiatric symptoms in military Veterans with a history of mild-to-moderate TBI.

Method: Participants included 140 Veterans (TBI=83, military controls [MC]=57) who underwent APOE genotyping (participants were blinded to APOE status) and were divided into e4+ (TBI=19, MC=16) and e4- (TBI=64, MC=41) groups. All participants underwent a comprehensive neuropsychological assessment, including completion of self-report measures assessing psychological distress. The primary outcome measures were the total score from the Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), and PTSD Checklist (PCL). Two-way analyses of variance were conducted to examine the effect of group (TBI vs. MC) and e4 status (e4+ vs. e4-) across symptom measures.

Results: There was a significant main effect of group across all symptom measures (TBI>MC; all p-values<. 001; np2=. 226-. 295), no main effect of e4 genotype (p=.173-.213, np2=. 011-.014), and a significant interaction of group by e4 genotype across all measures (p=.030-.041, np2=.030-.034). Specifically, for TBI participants, e4+ Veterans had significantly higher symptom scores than e4- Veterans (p=.008-.012, np2=.046-.050). For MC participants, e4 status had no effect (p=.541-.621, np2=.002-.003).

Conclusions: The results suggest a potentially meaningful relationship between APOE genotype and psychiatric distress following TBI, wherein the presence of an e4 allele conveys risk for increased symptomatology in the presence of neurological insult. Although findings are preliminary, this study furthers our understanding of how genetic factors influence response to TBI.

Abstracts to be presented in 2018:

Blast Exposure is Associated with Anterior Cortical Thinning in Veterans with Mild Traumatic Brain Injury

Alexandra L. Clark, Scott F. Sorg, Victoria C. Merritt, Katherine J. Bangen, Nicole Evangelista, Kelsey Holiday, Mark W. Bondi, Dawn M. Schiehser, & Lisa Delano-Wood

Objective: Many Veterans with mild traumatic brain injury (mTBI) are exposed to blast while on deployment. Blast exposure —even at low levels—has been linked to gray and white matter brain changes, yet few studies have examined associations with cortical thickness. Therefore, we sought to clarify whether regional differences in cortical thickness exist between those with mTBI who were and were not exposed to blast. We hypothesized that blast-exposure would be associated with increased cortical thinning, especially in vulnerable frontal regions where blast waves may concentrate.

Methods: 89 mTBI Veterans underwent neuroimaging, neuropsychological testing, and completed the post-traumatic-stress (PTSD) inventory. Participants (mTBI+blast, n=60; mTBI-blast, n=29) were compared on cortical thickness values derived from the following regions of interest (ROIs) using FreeSurfer: middle frontal gyrus (MFG), inferior frontal gryus (IFG), orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC).

Results: ANOVAs revealed the groups were comparable on basic demographics, psychiatric symptoms, and injury characteristics. ANCOVAs controlling for age, number of TBIs, and PTSD symptoms revealed that the mTBI+blast group showed thinner cortices of the bilateral MFG (*p*'s <.05) and right IFG ($p = .01$) relative to the mTBI-blast group. No significant differences between groups were observed for the right and left OFC and ACC (*p's* >.05). Across the sample, regression analyses adjusting for age and PTSD revealed that thinner MFG cortices were associated with poorer executive functioning (*p*'s < .05).

Conclusions: Findings suggest that Veterans with mTBI and blast exposure may be at especially high risk for negative brain and behavioral changes. Future longitudinal studies are needed in order to determine whether blast exposure exacerbates mTBI-related brain changes or independently negatively influences brain structure.

Hippocampal Volume Independently Predicts Subjective Memory Complaints in Mild Traumatic Brain Injury

Kelsey A. Holiday, Alexandra L. Clark, Scott Sorg, Michael Walsh, John Strom, Lisa Delano-Wood, & Dawn M. Schiehser

Objective: Veterans with mild traumatic brain injury (mTBI) frequently report memory deficits, which are not always supported by objective neuropsychological testing and are often related to psychiatric symptoms, such as PTSD. Given that neuroimaging allows for examination of

possible neural correlates of subjective memory complaints, the objective of our study was to examine the relationship between hippocampal volumes, objective memory performance, and PTSD symptoms on subjective memory complaints in Veterans with history of mTBI.

Participants and Methods: 64 Veterans with mTBI and optimal effort completed tests of objective verbal and visual memory (WMS-IV Logical Memory and Visual Reproduction), subjective memory (NSI-14), the PTSD Checklist, and structural MRI. FMRIB's Software (FSL) was used to obtain hippocampal as well as intracranial volume (ICV) estimates. Two hierarchical regression analyses were conducted to examine whether right or left hippocampal volume, visual or verbal memory performance, and/or PTSD symptoms predicted subjective memory complaints controlling for ICV and age.

Results: Regression analyses demonstrated that although objective memory performance was not associated with subjective memory complaints, left hippocampal volume was significantly and independently associated with subjective complaints (p=.001), with smaller volumes relating to greater complaint severity; results revealed a similar trend for the right hippocampal volume (p=.057). As expected, PTSD strongly predicted subjective memory complaints (p's<.001).

Conclusions: In mTBI Veterans, subjective memory complaints are best accounted for by PTSD symptoms and hippocampal volume, but not objective performance. Importantly, smaller hippocampal volumes are independently associated with greater subjective memory severity, suggesting a neural basis of memory complaints in mTBI not accounted for by PTSD or objective memory performance. Future research is needed to elucidate the behavioral manifestations of subjective memory complaints in mTBI.

APOE-ε4 Genotype Modifies the Relationship Between TBI History and Neuropsychological Performance in Military Veterans

Merritt, V. C., Clark, A. L., Sorg, S. F., Evangelista, N. D., Werhane, M., Bondi, M. W., Schiehser, D. M., & Delano-Wood, L.

Objective: Although genetic differences may contribute to the varied cognitive outcomes often observed after traumatic brain injury (TBI), the influence of genetic factors on neuropsychological performance following TBI remains poorly understood. We therefore investigated the influence of APOE polymorphisms on neurocognitive functioning in Veterans with post-acute TBI.

Participants and Methods: 121 Veterans (mild TBI=59, moderate TBI=10; military controls [MC]=52) underwent neuropsychological assessment and APOE genotyping. Veterans were divided into ε4+ (TBI=17, MC=14) and ε4- (TBI=52, MC=38) groups. Two composite scores were computed from 21 norm-referenced variables—a memory index (α=.84) and a speed index (α=.88), and the total number of impaired scores (>1.5 SD below mean) for each participant was calculated. Two-way ANOVAs were conducted to examine the effect of group (TBI vs. MC) and ε4 status (ε4+ vs. ε4-) on cognition.

Results: The main effect of ε4 genotype was significant for both cognitive composites (ε4+<ε4-; *p*=.02-.03) and the main effect of group approached significance (TBI<MC; *p*=.05-.08). Compared to the TBI ε4- group, the TBI ε4+ group performed more poorly on the memory composite (*p*=.02), and there was a trend in the same direction for the speed index (*p*=.05). There was no effect of ε4 status across cognitive indices in the MC group (*p*>.05). Within the TBI group, ε4+ Veterans showed a greater number of impaired scores than ε4- Veterans (*p*=.01), and 82% of the TBI ε4+ group had ≥1 impaired score compared to 44% of the TBI ε4- group (*p*=.01).

Conclusions: Results suggest that APOE-ε4 genotype contributes to poorer memory and processing speed in Veterans with TBI. Our findings extend prior work by suggesting that APOE-ε4 genotype is associated with cognitive impairment even well beyond the acute phase of injury. Future work is needed to elucidate the underlying brain-based mechanisms of ε4 allelic effects on cognitive and clinical outcomes following TBI.

Subjective Ratings of Retrospective and Prospective Memory Difficulties in Veterans with Mild Traumatic Brain Injury

Scott F. Sorg, Alexandra L. Clark, Laura M. Campbell, Madeleine Werhane, Kelsey A. Holiday, Victoria Merritt, Amy J. Jak, Dawn M. Schiehser & Lisa Delano-Wood

Objective: The validity of subjective memory complaints in mild traumatic brain injury (mTBI) remains an important clinical consideration especially in the context of co-morbid psychiatric symptoms (PTSD). In this study, we assessed memory complaints in Veterans with mTBI and evaluated if an effect of mTBI persisted after controlling for PTSD status, and investigated specific memory-type associations with injury severity and performance on objective neuropsychological tests.

Methods: 98 Veterans (mTBI=65, Military Controls [MCs]=26) completed a structured TBI history interview, the Prospective-Retrospective Memory Questionnaire (PRMQ) with Prospective (PM) and Retrospective Memory (RM) subscales, the PTSD Checklist, and objective tests of memory (Logical Memory and CVLT-II) and executive functions (Wisconsin Card Sorting Test and D-KEFS Trails) with optimal effort testing.

Results: Compared to MCs, the mTBI group endorsed higher PTSD symptoms and PRMQ scores across PM and RM subscales (*p*'s<.01). Within the mTBI group, PM complaints were greater in Veterans reporting loss of consciousness (n=43) relative to those reporting no loss of consciousness (n=22, *p*<.05), but there were no significant differences in RM ratings. There were no differences in age, number of TBIs, or mode of injury between LOC and AOC groups. In the mTBI group, objective memory test scores were associated with RM complaints (r=-.34 – -.40, *p*'s<.01), but not PM. Executive function test scores were associated with both RM and PM (r=-.26 – -.40, *p*'s<.05). All group comparisons and associations remained significant after

statistically adjusting for PTSD status.

Conclusions: Results found associations between mTBI status and subjective memory complaints were independent of co-morbid psychiatric status in Veterans with head injury histories. Findings further suggest greater injury severity contributes to worse subjective PM complaints and suggest dissociable associations with object test performances between RM and PM ratings.

Associations Between Time Since Injury and Cognitive Recovery Across Mechanism of Traumatic Brain Injury: Preliminary Evidence for Protracted Cognitive Recovery in Blast-Related Neurotrauma

John Strom, Scott F. Sorg, Kelsey A. Holiday, Alexandra L. Clark, Madeleine Werhane, Mark W. Bondi, Dawn M. Schiehser, Lisa Delano-Wood

Objective: Although recent studies have suggested that blast force traumatic brain injury (TBI) may cause damage to brain regions typically unaffected by blunt force TBI, little is known about whether cognitive recovery differs across mechanism of injury (blast versus blunt force TBI). We therefore investigated associations between time since injury and TBI mechanism on executive function performance in a group of well-characterized Veterans with history of mild to moderate TBI.

Participants and Methods: 214 Veterans (blast-TBI [n=44], blunt-TBI [n=82], or non-TBI military controls [MCs: n=88]) completed a clinical interview, neuropsychological testing, and psychiatric questionnaires. Groups were compared using the following executive function (EF) tests: WAIS IV Digit Span, WASI Block Design, D-KEFS Verbal Fluency, Trail Making Test, and Color Word Interference.

Results: As expected, compared to MCs, both TBI groups performed significantly more poorly across EF tests (all *p*-values < .05). Regression analyses adjusting for age, severity of injury, and PTSD symptoms, showed that greater time since injury was significantly related to higher (better) EF scores within the blast-TBI group but not blunt-TBI group (all *p*–values < .02). In contrast, no such effects of time since injury on cognition were found in the blast-TBI group (all p-values > .05).

Conclusions: Results showed that increased time since injury was related to better performance on tasks of executive functioning in Veterans with blunt force but not blast-related TBI. Importantly, these findings were independent of PTSD symptomatology, and they were not explained by injury severity. Taken together, findings suggest that blast-exposed Veterans may evidence a more protracted recovery of cognitive functions in the aftermath of TBI. Future studies are needed in order to examine whether and how brain regions may be differentially affected across mechanism of injury, and how other variables (e.g., genetic influences) may modify any observed relationships.

Regional gray matter volumetric differences predict fatigue symptoms in veterans with mild traumatic brain injury.

Michael J. Walsh, Scott F. Sorg, Alexandra L. Clark, Kelsey Holiday, Nicole D. Evangelista, Lisa Delano-Wood, Dawn M. Schiehser

Objective : Fatigue is a common complaint of military Veterans following mild traumatic brain injury (mTBI); however, the etiologic basis of fatigue remains poorly understood. Given that studies across other neurological disorders (e.g., multiple sclerosis, Parkinson's disease) have shown that greater levels of fatigue are associated with reductions in gray matter volume, we sought to clarify whether gray matter volumes are associated with self-reported fatigue in Veterans with history of mTBI.

Participants and Methods: 106 Veterans (mTBI = 53, demographically-matched Veteran Controls [VCs] = 53) completed the Modified Fatigue Impact Scale (MFIS) and underwent structural MR imaging. Cortical and subcortical gray matter volumes were extracted from parcellated and segmented regions of interest (ROIs) using FreeSurfer. Analyses of Variance were conducted to evaluate group differences in MFIS scores and regional grey matter volumes. A hierarchical regression was conducted to determine whether there was a significant relationship between fatigue and significant regional gray matter volumes.

Results : Compared to VC, mTBI Veterans endorsed significantly higher levels of fatigue on the MFIS ($p < .001$) and evidenced reduced right nucleus accumbens (NA; $p < .001$) volume as well as trend reduction of volume in the right rostral anterior cingulate (rACC; $p = .057$). Reduced right NA volume ($p = .001$) significantly predicted greater levels of fatigue, while the right rACC ($p = .064$) showed a similar trend.

Conclusions : Our results demonstrated that higher levels of self-reported fatigue were predicted by reduced regional gray matter volumes in participants with a history of post-acute mTBI. These findings suggest that damage to specific brain regions may play a role in the manifestation of fatigue symptoms in the context of mild neurotrauma. Further studies must determine whether regional gray matter volumes are differentially associated with cognitive and physical fatigue, and whether mechanism of injury plays a role in outcomes.

Apolipoprotein E-ε4 Genotype and Pulse Pressure Interact to Affect Cortical Thickness in Brain Regions Vulnerable to Neurodegeneration in Veterans with Mild Traumatic Brain Injury

Madeleine Werhane, Kristina Lapira, Alexandra L. Clark, Nicole D. Evangelista, Scott F. Sorg, Mark W. Bondi, Dawn Schiehser, & Lisa Delano-Wood

Objective*:* Although possession of the APOE-ε4 allele has been repeatedly linked to poor cognitive and brain outcomes in mild traumatic brain injury (mTBI) samples, it remains unclear how it might interact with vascular risk factors in the expression of pathological brain changes in

the aftermath of neurotrauma. Thus, we explored the independent and interactive effects of elevated vascular risk and APOE-ε4 status on cortical thinning in brain regions vulnerable to later neurodegenerative changes in Veterans with history of mTBI.

Participants and Methods: 48 Veterans (mean age=32 years) with history of mTBI underwent structural magnetic resonance imaging (MRI), APOE genotyping, and blood pressure (BP) assessment. Pulse pressure (PP), an index of arterial stiffening, was derived from blood pressure (BP) values (systolic – diastolic BP). Multiple regression was used to assess whether genetic status predicted cortical thickness in FreeSurfer-derived regions of interest (ROIs).

Results: Multiple regression, adjusting for age and PTSD severity, showed that APOE-ε4 positivity modified the relationship between PP and cortical thickness in the medial orbitofrontal (p=.023) and precuneus (p=.045) regions. Specifically, for ε4 carriers, lower PP values were associated with greater cortical thinning in these regions. No such relationship was observed in non-ε4 carriers.

Conclusions: Our results revealed that, in our sample of relatively young Veterans with a history of mTBI, PP and APOE- ε4 status interact to affect cortical thickness in regions known to be affected early in the context of neurodegenerative disorders such as Alzheimer's disease. Specifically, even after adjusting for age and PTSD symptom severity, APOE-ε4 carriers with lower PP demonstrated reduced cortical thickness in orbitofrontal and precuneus regions. These results add to a growing literature highlighting the complexity of vascular and genetic implications on AD pathology following neurotrauma.

Quanitative Tractography and Volumetric MRI in Blast and Blunt Force TBI: Predictors of Neurocognitive and Behavioral Outcome

W81XWH-10-2-0169

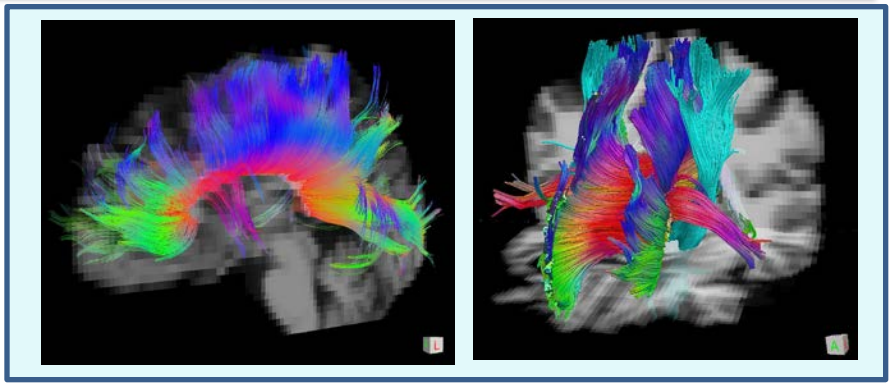
PI: Lisa Delano-Wood **Org:** Veterans Medical Research Foundation **Award Amount:** $1,217,829

Study/Product Aim(s)

- Use DTI to examine whether differences in cognitive outcome are related to mechanisms of injury and hippocampal volumes and white matter integrity
- Determine whether MR variables are associated with clinical outcome and whether there are group differences by mechanism of injury
- Use of novel MRI methods to characterize white matter changes across TBI subtypes
- Identify those at highest risk for poor outcomes and gain knowledge about potential interventions
- Investigate potential contribution of genetic factors in neurobehavioral outcome after TBI

Approach

The following groups are recruited through the VA San Diego Polytrauma and Neuropsychology Units: (a) a blast-related injury group; (b) a mechanical force injury group; and (c) an orthopedic control group. Participants will be recruited from consecutive admissions for inpatient or outpatient treatment of TBI or orthopedic injuries. As part of their enrollment in the study, all participants will undergo detailed cognitive, neurologic, genetic, and psychosocial/clinical evaluations. All participants undergo genotyping with a swab of the inside of the cheek to obtain a saliva sample to determine each participant's genetic (apolipoprotein ε4 [APOE ε4) and neurotrophic factors (brain-derived neurotrophic factor [BDNF]). Participants will be scanned in a 3-Tesla GE Signa Infinity MRI scanner Each participant will receive a 3D whole-brain T2-weighted image series This protocol provides multi-spectral information used in co-registration and skull-stripping, and they are optimized for gray, white and CSF tissue segmentation. Additionally, all participants will receive a 61-direction DTI sequence



By employing quantitative Diffusion Tensor Imaging (DTI), we see the result of DTI in conjunction with a tract-tracing algorithm displaying the corpus callosum. The corpus callosum is by far the largest fiber bundle in the human brain, allowing functional intergration and communication between the two hemispheres. We use this advanced and sensitive neuroimaging method in order to relate important injury severity variables that correspond to TBI neuropathology.

Activities CY	10	11	12	13	14	15	16	17
Obtain IRB approval								
Participant Recruitment								
Data Analyses (MRI, cognitive)								
Manuscript Preparation								
Estimated Budget ($K)	$254	$342	$350	$272				

Goals/Milestones

CY10 Goal – Recruitment and Data Acquisition
☐ Recruit and enroll eligible participants
☑Data collection
☑ Obtain IRB approval
CY11 Goals – Recruitment and Data Acquisition cont'd
☐ Continued participant recruitment
☑Genetic testing and MRI acquisition
CY12 Goal – Preliminary Data Analyses
☑Analyze existing cognitive data
☑MRI processing
☑Manuscript preparation
CY13-17 Goal – Advanced Data Analyses and Publication
Recruit and enroll eligible participants
Continued participant recruitment
Publish and present findings

Comments/Challenges/Issues/Concerns

- The study has experienced difficulty in recruitment. Numbers are not as high as we would like, which necessitated the request for cost-free extension. We were granted this no-cost extension on 9/10/14.
- We were granted a second no-cost extension on 9/29/15, and a third on 9/30/16.

Budget Expenditure to Date

Projected Expenditure: $1,217,829 Actual Expenditure: $1,215,975