

Mild traumatic brain injury is associated with reduced cortical thickness in those at risk for Alzheimer's disease

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Moderate-to-severe traumatic brain injury is one of the strongest environmental risk factors for the development of neurodegenerative diseases such as late-onset Alzheimer's disease, although it is unclear whether mild traumatic brain injury, or concussion, also confers risk. This study examined mild traumatic brain injury and genetic risk as predictors of reduced cortical thickness in brain regions previously associated with early Alzheimer's disease, and their relationship with episodic memory. Participants were 160 Iraq and Afghanistan War veterans between the ages of 19 and 58, many of whom carried mild traumatic brain injury and post-traumatic stress disorder diagnoses. Whole-genome polygenic risk scores for the development of Alzheimer's disease were calculated using summary statistics from the largest Alzheimer's disease genome-wide association study to date. Results showed that mild traumatic brain injury moderated the relationship between genetic risk for Alzheimer's disease and cortical thickness, such that individuals with mild traumatic brain injury and high genetic risk showed reduced cortical thickness in Alzheimer's disease-vulnerable regions. Among males with mild traumatic brain injury, high genetic risk for Alzheimer's disease was associated with cortical thinning as a function of time since injury. A moderated mediation analysis showed that mild traumatic brain injury and high genetic risk indirectly influenced episodic memory performance through cortical thickness, suggesting that cortical thinning in Alzheimer's disease-vulnerable brain regions is a mechanism for reduced memory performance. Finally, analyses that examined the apolipoprotein E4 allele, post-traumatic stress disorder, and genetic risk for schizophrenia and depression confirmed the specificity of the Alzheimer's disease polygenic risk finding. These results provide evidence that mild traumatic brain injury is associated with greater neurodegeneration and reduced memory performance in individuals at genetic risk for Alzheimer's disease, with the caveat that the order of causal effects cannot be inferred from cross-sectional studies. These results underscore the importance of documenting head injuries even within the mild range as they may interact with genetic risk to produce negative long-term health consequences such as neurodegenerative disease.

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Abbreviations: CVLT-II = California Verbal Learning Test-Second Edition; GWAS = genome-wide association study; PSQI = Pittsburgh Sleep Quality Index; PTSD = post-traumatic stress disorder; SNP = single nucleotide polymorphism; TBI = traumatic brain injury; WTAR = Wechsler Test of Adult Reading.

Introduction

Traumatic brain injury (TBI) results from an external force applied to the head that causes alterations in consciousness such as dizziness, amnesia, or loss of consciousness. TBI of varying levels of severity has been associated with neural and cognitive changes that can persist chronically, years after the initial injury (Kraus *et al.*, 2007; Hayes *et al.*, 2015; Miller *et al.*, 2016). Of concern for the millions of Americans injured each year, TBI is one of the strongest environmental risk factors for the development of neurodegenerative diseases such as late-onset Alzheimer's disease and chronic traumatic encephalopathy. The link between TBI and Alzheimer's disease has been studied for several decades, with two meta-analyses confirming enhanced risk for Alzheimer's disease following TBI, particularly among males (Mortimer *et al.*, 1991; Fleminger *et al.*, 2003). However, the mechanisms that instantiate risk for Alzheimer's disease after TBI are unknown. Researchers have noted a number of common features associated with both TBI and Alzheimer's disease including inflammatory and apoptotic (i.e. programmed cell death) processes, along with the accumulation of proteins that contribute to cell death. For instance, Uryu *et al.* (2002, 2007) demonstrated in both mouse models and in human subjects that TBI is associated with amyloid- β , α -synuclein, and tau, three pathological markers of Alzheimer's disease.

However, the aforementioned neurodegenerative markers have primarily been observed in moderate-to-severe TBI (Johnson *et al.*, 2012) and some have suggested that risk for dementia following TBI increases with severity (Plassman *et al.*, 2000). Although there is some preliminary evidence that mild TBI is also associated with increased risk of dementia and its biomarkers in older adults (Gardner *et al.*, 2014), the evidence has been inconsistent and has led some to suggest that mild TBI alone, without the presence of additional risk factors, may be insufficient to develop dementia (Bigler, 2013). As the majority of TBIs sustained in civilian and military contexts are within the mild range (Cassidy *et al.*, 2004; Terrio *et al.*, 2009), the relationship between mild TBI and dementia risk is an important issue requiring further study.

In addition to environmental risks for Alzheimer's disease (e.g. TBI), there is abundant evidence for genetic

contributions to the disease. In light of the strong heritability of late-onset Alzheimer's disease (60–80%; Gatz *et al.*, 2006), an important question is whether TBI moderates the genetic risk for Alzheimer's disease. Studies to date have focused on TBI in individuals carrying the apolipoprotein (*APOE*) E4 allele, with inconsistent results. Some studies have found poorer outcomes in E4 carriers following TBI (Jordan *et al.*, 1997; Friedman *et al.*, 1999), whereas others have not, or have found that E4 carriers were less likely to have dementia following head injury (Guo *et al.*, 2000; Jellinger *et al.*, 2001; Chamelian *et al.*, 2004).

Interest has grown in the calculation of polygenic risk scores to index genetic risk since the publication of a seminal Psychiatric Genomics Consortium study that used a polygenic score to explore the genetic architecture of schizophrenia and bipolar disorder (Purcell *et al.*, 2009). A polygenic risk score uses the results from a genome-wide association study (GWAS) of a particular trait to compute a genetic propensity score for that trait in a new sample or cohort with available genome-wide genotype data. Recent work suggests that polygenic risk scores for Alzheimer's disease are better predictors of Alzheimer's disease than are single candidate variants such as *APOE*. It has been shown that genome-wide single nucleotide polymorphism (SNP) analysis explained 33% of the phenotypic variance in Alzheimer's disease in comparison to 6% explained with *APOE*, and the 8% of variance explained by the combination of 11 other Alzheimer's disease risk SNPs (Ridge *et al.*, 2013). Recently, Sabuncu *et al.* (2012) demonstrated that healthy subjects with higher polygenic risk scores derived from an Alzheimer's disease GWAS showed reduced cortical thickness in areas affected most by Alzheimer's disease (Sabuncu *et al.*, 2011). These results suggest that polygenic risk scores may serve as predictors of prodromal Alzheimer's disease-related brain pathology.

In the present study, we hypothesized that individuals with both mild TBI and high polygenic risk for Alzheimer's disease would be the most likely to show reduced cortical thickness in previously identified Alzheimer's disease-vulnerable regions (Sabuncu *et al.*, 2011). Further, we investigated the hypothesis that reduced cortical thickness in these same regions reflected a neurodegenerative process by examining associations between cortical thickness and years passed since the mild TBI.

Finally, we hypothesized that reduced cortical thickness would be associated with lower delayed memory performance, a neuropsychological domain particularly sensitive in detecting Alzheimer's disease. In additional analyses, we examined specificity of the main findings by considering whether repetitive mild TBI, the *APOE* E4 allele, and polygenic risk for mental illnesses were also significant predictors of cortical thickness. We examined these hypotheses in a sample of Iraq and Afghanistan War veterans exposed to a range of mild TBI events during their lifetimes, including direct forceful contact of the head and blast-related mild TBI experienced in combat. Among those exposed to TBI, the sample was limited to individuals with mild severity in order to examine potential links between this most common type of TBI and markers of neurodegenerative disease. We sought to shed light on whether there are markers of prodromal neurodegeneration (i.e. reduced cortical thickness in Alzheimer's disease-vulnerable regions) in a younger cohort of veterans who were exposed to TBI (i.e. those who served in the Iraq and Afghanistan Wars). Although it is known that TBI in older military personnel is associated with a 60% increase in the risk of developing neurodegenerative disease (Barnes *et al.*, 2014), detection of neurodegenerative markers in a younger cohort would have important implications for development of interventions to inhibit TBI-induced neurodegeneration as well as long-term care for these veterans. Finally, we studied the influence of post-traumatic stress disorder (PTSD) during the lifespan on Alzheimer's disease-related cortical thinning, a common comorbidity in the veteran cohort, which shares behavioural symptoms with neurodegenerative disease (Omalu *et al.*, 2011).

Materials and methods

Participants

The final sample included 160 white non-Hispanic veterans of Operations Enduring Freedom, Iraqi Freedom, and New Dawn who were consecutively enrolled into the Translational Research Center for TBI and Stress Disorders (TRACTS) at the Jamaica Plain Division of the Veterans Affairs Boston Healthcare System. Participants were recruited from throughout the Boston Metropolitan area through outreach military community events (e.g. Yellow Ribbon events). We used all available TRACTS data that met our selection criteria. We first identified 258 white, non-Hispanic subjects to avoid population stratification effects. Of these, 55 subjects were missing genotype data, an additional 29 were missing neuroimaging data, eight were missing clinical assessment data, and six individuals were excluded because they had moderate and/or severe TBI. Demographics as a function of mild TBI status in the entire sample are shown in Table 1 and characteristics of the mild TBI sample, including nature of injury, and duration of alterations in mental status, post-traumatic amnesia, and loss of consciousness are shown in Table 2. All subjects experienced alterations in mental status, 76% experienced

post-traumatic amnesia, and 57% of the sample experienced loss of consciousness. The groups did not differ significantly in age, sex, or Wechsler Test of Adult Reading-estimated IQ (WTAR; Wechsler, 2001), but they did differ in the presence of lifetime PTSD and sleep disturbance. Additional details regarding inclusion/exclusion criteria for the sample are included in the Supplementary material. Study procedures were approved by the VA Boston Institutional Review Board and all participants provided written informed consent consistent with the Declaration of Helsinki.

MRI acquisition and processing

Participants were scanned on a Siemens 3 T TIM Trio located at the Jamaica Plain Division of VA Boston Healthcare System. Two 3D magnetization-prepared rapid gradient-echo (MP-RAGE) scans were acquired in the sagittal plane and averaged to create a single high contrast-to-noise image (voxel size = 1 mm³, T₁ = 1000 ms, repetition time = 2530 ms, echo time = 3.32 ms). Automated cortical reconstruction and volumetric segmentation were performed with the FreeSurfer image analysis suite (version 5.0), which is available for download online (<http://surfer.nmr.mgh.harvard.edu/>). See Supplementary material for details regarding imaging analysis with FreeSurfer.

Cortical thickness values were extracted from seven regions including the entorhinal cortex, temporopolar cortex, lateral temporal cortex, inferior parietal cortex, inferior parietal sulcus, posterior cingulate cortex, and inferior frontal cortex (Fig. 1). These regions of interest were selected because collectively, they have shown the greatest bilateral cortical thinning in early Alzheimer's disease in comparison to healthy older adults (Sabuncu *et al.*, 2011). Region of interest masks were downloaded from the Open Access Series of Imaging Studies (OASIS) dataset, which is publically available with FreeSurfer. Regions of interest were registered to each individual subject's cortical representation via surface-based registration and cortical thickness values were extracted for each subject. Values were averaged across left and right hemispheres and then averaged to create a single cortical thickness score across the seven regions (Sabuncu *et al.*, 2011). An additional seven FreeSurfer regions of interest were selected to serve as control regions for the putative Alzheimer's disease-vulnerable regions of interest. Selection of these regions was semi-random with the constraint that, similar to the Alzheimer's disease-vulnerable regions, regions within the frontal, temporal, and parietal lobes would be represented. These included the middle temporal gyrus, lateral occipital gyrus, paracentral gyrus, postcentral gyrus, lingual gyrus, frontal pole, and the insula.

Clinical and cognitive assessment

TBI history was assessed using the Boston Assessment of TBI-Lifetime (BAT-L; Fortier *et al.*, 2014), which was administered by a doctoral-level psychologist with extensive training in neuropsychology. The BAT-L is a semi-structured interview based on the Department of Defense diagnostic criteria of TBI and captures self-reported history of TBI experienced pre-, during, and post-military deployment. Mild TBI was defined as loss of consciousness lasting <30 min, and/or an altered mental status lasting <24 h, and/or a period of post-traumatic amnesia lasting <24 h. Additional details regarding TBI assessment are included in the Supplementary material.

Table 1 Demographic and clinical characteristics

Variable	Total (<i>n</i> = 160)	No lifetime mild TBI (<i>n</i> = 55)	Lifetime mild TBI (<i>n</i> = 105)	Group comparison
Age in years, mean (SD)	31.36 (8.4)	32.85 (8.9)	30.57 (8.1)	$t(158) = 1.64, P = 0.103$
Males, <i>n</i> (%)	149 (93.1)	51 (92.7)	98 (93.3)	$\chi^2(1) = 0.02, P = 0.89$
WTAR Estimated IQ, mean (SD)	102.12 (19.8)	103.42 (11.9)	101.45 (22.9)	$t(158) = 0.60, P = 0.55$
APOE $\epsilon 4$ status <i>n</i> (%)	37 (23.1)	10 (18.2)	27 (25.7)	$\chi^2(1) = 1.15, P = 0.28$
Lifetime PTSD diagnosis <i>n</i> (%)	112 (70.0)	28 (50.9)	84 (80.0)	$\chi^2(1) = 14.55, P < 0.001$
PSQI, <i>n</i> (%)	113 (70.6)	31 (56.4)	82 (78.1)	$\chi^2(1) = 8.22, P = 0.004$

Table 2 Injury characteristics of the mild TBI sample

Lifetime mild TBI (<i>n</i>, %)	
Total	105, 100%
Military-related mild TBI	56, 53%
More than one mild TBI	49, 47%
Most severe mild TBI (minutes, SD)	
Altered mental status	126.12, 438.97
Post-traumatic amnesia	25.44, 131.26
Loss of consciousness	2.37, 4.96
Nature of injury (most severe)	
Military mild TBI	
Blast	25
Fall	5
Motor vehicle accident	4
Physical assault	2
Sports-related injury	4
Other ^a	5
Total	45
Pre-deployment mild TBI	
Fall	7
Motor vehicle accident	10
Physical assault	7
Sports-related injury	23
Other ^a	6
Total	53
Post-deployment mild TBI	
Fall	1
Motor vehicle accident	2
Physical assault	1
Sports-related injury	2
Other ^a	1
Total	7
Nature of injury Totals (<i>n</i> , %)	
Blast	25, 24%
Fall	13, 12%
Motor vehicle accident	16, 15%
Physical assault	10, 10%
Sports-related injury	29, 28%
Other ^a	12, 11%

^aOther injuries include: hit by blunt object (e.g. pole, bar, vehicle, ceiling). Most severe nature of injury is reported, but many individuals had multiple types of injuries. Most severe mild TBI was determined by length of loss of consciousness, or post-traumatic amnesia, or altered mental status if the subject did not experience loss of consciousness or post-traumatic amnesia.

Lifetime PTSD was assessed with the Clinician-Administered PTSD Scale (CAPS; Blake *et al.*, 1995), a structured diagnostic interview that is the gold standard for assessment of the disorder. The CAPS assessment was performed by doctoral level

psychologists with advanced training in psychological assessment. On this interview, each DSM-IV PTSD criterion is assessed with two CAPS sub-items, one that reflects the frequency of the symptom on a 0–4 scale and one that reflects the intensity of the symptom on a 0–4 scale. As with TBI, PTSD diagnosis was decided via consensus meeting of doctoral-level psychologists. Sleep disturbance was measured by the Pittsburgh Sleep Quality Index (PSQI; Buysse *et al.*, 1989) and defined as a global score >5 on the PSQI. Premorbid IQ was estimated using the WTAR. This measure contains 50 irregularly spelled words of increasing difficulty to pronounce. The ability to perform this test remains unchanged following disease and injury. Episodic memory performance was assessed using the long-delay free recall score of the California Verbal Learning Test-Second Edition (CVLT-II; Delis *et al.*, 2000). Long delay list learning tasks have been found to be the most sensitive to early Alzheimer's disease (Welsh *et al.*, 1992; Weintraub *et al.*, 2012). One subject was missing this measure, and therefore analyses with the CVLT-II had a total *n* = 159.

DNA genotyping and polygenic risk score computation

APOE genotyping was conducted by the Pharmacogenomics Analysis Laboratory at the Central Arkansas Veterans Healthcare System, Little Rock, Arkansas. A detailed description of the genotyping protocol and generation of genome-wide genotypes is presented in the Supplementary material.

A polygenic risk score is a weighted average of genome-wide genotype data. The weights are the beta values estimated from the GWAS (or log odds ratios) if the GWAS was of a dichotomous trait. The polygenic risk score is the sum of these weights times the additively coded genotypes in the target population (0, 1, 2 coding). Polygenic scores are usually computed based on all SNPs from the GWAS under a specified *P*-value threshold such as $P < 0.05$ or $P < 0.5$. As there is as of yet no *a priori* way to identify which cut-off yields the most predictive scores, scores are usually computed over multiple *P*-value thresholds, often with a multiple-testing correction to judge their significance, and then the most predictive is used in further calculations or validated in an additional sample. In addition, the SNP sets are often 'trimmed' for linkage disequilibrium (i.e. non-independence between variants) to avoid the inclusion of redundant information in score calculation. In the present study, the polygenic risk scores for Alzheimer's disease were computed based on summary results from the International Genomics of Alzheimer's Disease Project (IGAP) GWAS (Lambert *et al.*, 2013) as downloaded from

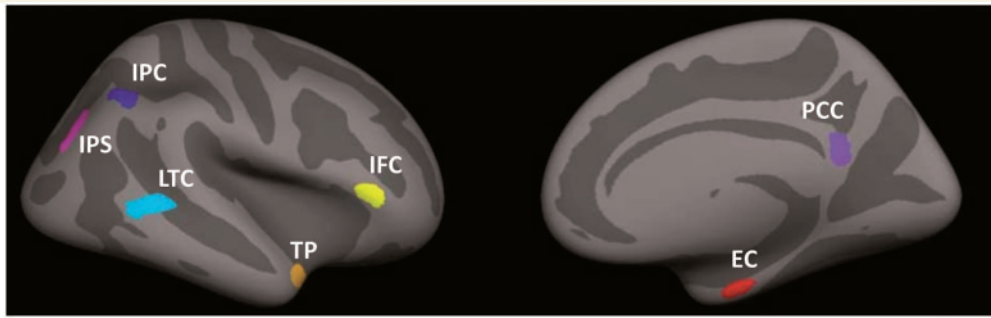


Figure 1 Seven Alzheimer's disease vulnerable regions of interest. IPC = inferior parietal cortex; IPS = inferior parietal sulcus; LTC = lateral temporal cortex; IFC = inferior frontal cortex; TP = temporopolar cortex; PCC = posterior cingulate cortex; EC = entorhinal cortex. Figure reproduced with permission from Sabuncu *et al.* (2011).

http://web.pasteur-lille.fr/en/recherche/u744/igap/igap_download.php. Poorly imputed SNPs (Impute2 quality < 0.50) and rare SNPs (minor allele frequency < 0.01) were excluded from risk score calculation. Prior to imputation, the SNP panel was trimmed for linkage disequilibrium using PLINK's 'clumping' procedure (r^2 threshold of 0.2 in a 500 kb window based on linkage disequilibrium patterns in the 1000 Genomes EUR sample). Polygenic scores were computed assuming six different P -value thresholds: $P < 0.05$, $P < 0.10$, $P < 0.20$, $P < 0.30$, $P < 0.40$, and $P < 0.50$. As a control, schizophrenia and depression polygenic risk scores were also computed from the Psychiatric GWAS Consortium results using the same methodology (Ripke *et al.*, 2013a, b) (<https://www.med.unc.edu/pgc/downloads>).

Statistical approach

Regression models examining mild TBI, PTSD, genetic risk for Alzheimer's disease and cortical thickness

Data were analysed using a series of linear regression models. Main effects of mild TBI and Alzheimer's disease polygenic risk score models were fit by which the average cortical thickness across the seven Alzheimer's disease-vulnerable regions was regressed onto the polygenic risk score at each of the six polygenic risk score P -value thresholds. Covariates for all models and follow-up tests included age, sex, WTAR, PSQI, and lifetime PTSD. Similar models were used to assess the mild TBI by polygenic risk score interaction, again run for each of the six P -value thresholds. Corrected significance for main effects and interactions was determined using Monte-Carlo null simulation with 10 000 replicates in which the genetic data were randomly permuted between subjects. This analysis imposed strict multiple-testing control while taking into account the correlations between the six polygenic risk score P -value threshold variables.

We selected the polygenic score threshold value that showed the strongest interaction effect with mild TBI on cortical thickness. All subsequent analyses used this threshold value. To parse the relative effects of the polygenic score, mild TBI and covariates, a series of *post hoc* models were fit using a three-step hierarchical regression analysis. In the first model, nuisance covariates (age, sex, WTAR, PSQI, lifetime PTSD

diagnosis) were entered. In the second model, the main effects of polygenic risk and mild TBI diagnosis were entered. Finally, the polygenic risk by mild TBI interaction term was entered into the third model. The results reported below refer to the output of Model 3 as we were most interested in the mild TBI by polygenic risk interaction. As there were only 11 female veterans in the entire dataset (seven with mild TBI), linear models were repeated including males only. Although education may also be entered as a covariate due to its association with onset of dementia in some studies, we selected premorbid IQ (i.e. WTAR) as a covariate due to evidence that when both premorbid IQ and education are entered into the model, premorbid IQ has a greater association with dementia than education (Pavlik *et al.*, 2006; Doody *et al.*, 2010). We further note that in this sample, education is not a significant predictor of cortical thickness, nor does its inclusion as a covariate change the results.

As there is evidence for higher risk of neurodegeneration following repetitive mild TBI (see Blennow *et al.*, 2012), we also examined whether number of mild TBIs sustained over the lifespan moderated the association between polygenic risk for Alzheimer's disease and cortical thickness (this model was limited to participants with TBI). To examine whether PTSD influenced the relationship between genetic risk and cortical thickness, the regression model included main effects for lifetime PTSD, polygenic risk for Alzheimer's disease, and their interaction.

Cortical thickness as a function of time since injury

To examine the hypothesis that genetic risk is associated with accelerated cross-sectional cortical thinning, we calculated the amount of time (in years) that had elapsed since the last reported TBI (age at study – age at most recent mild TBI). We added the interaction of this time variable and polygenic risk to our linear regression model (this model was also limited to participants with TBI).

Examining the effect of APOE and other Alzheimer's disease-related single nucleotide polymorphisms

Linear models were run to examine the main effect of APOE (E4 carriers, $n = 37$; non-E4 carriers, $n = 123$) and the mild TBI by APOE interaction on cortical thickness. Additional models assessed the top five SNPs that reached genome-wide significance for genetic risk for Alzheimer's disease from

Lambert *et al.* (2013) including rs6656401 in *CR1*, rs6733839 in *BIN1*, rs9331896 in *CLU*, rs10792832 in *PICALM*, and rs4147929 in *ABCA7*.

Effects in individual Alzheimer's disease-vulnerable brain regions and specificity of hypotheses

To examine to extent to which the interaction between mild TBI and genetic risk was associated with any particular region of interest or hemisphere among the seven Alzheimer's disease-vulnerable regions, we ran a three-step hierarchical regression model (covariates in step 1; main effects of mild TBI and polygenic risk in step 2; interaction term in step 3) for each of 14 brain regions (each of the seven Alzheimer's disease-vulnerable regions, left and right separately). To correct for multiple comparisons across the 14 hierarchical regression analyses, significance for main effects and interactions was determined using Monte-Carlo null simulation with 10 000 replicates.

To determine specificity of the Alzheimer's disease-vulnerable cortical thickness findings, we examined mild TBI and genetic associations with seven control regions of interest (see 'MRI acquisition and processing' section for details). Further, to determine the specificity of the polygenic risk for Alzheimer's disease scores, we examined the interaction between mild TBI and a depression polygenic risk score, and mild TBI and a schizophrenia polygenic risk scores to predict cortical thickness in Alzheimer's disease-vulnerable regions.

Moderated mediation analysis

To examine the relationships among mild TBI, polygenic risk, and memory performance, a regression-based path analysis was performed estimating and probing interactions and conditional indirect effects in a moderated mediation model using the PROCESS tool (Hayes, 2013) as implemented in SPSS v. 21. In this model, we examined evidence that the strength of the relationship between genetic risk and memory performance through cortical thickness is conditional on the value of a moderator (lifetime mild TBI). A detailed description of the moderated mediation model is available in the Supplementary material.

Results

Mild TBI but not PTSD moderates the relationship between genetic risk for Alzheimer's disease and cortical thickness

Regression models indicated a significant main effect of polygenic risk for Alzheimer's disease, with the strongest association occurring for the polygenic score computed using the $P = 0.2$ threshold (see Supplementary Table 1 for results at each polygenic risk P -value threshold). As predicted, there was a significant mild TBI by Alzheimer's disease polygenic risk (maximum effect with the $P = 0.2$ threshold) interaction whereby individuals with both mild TBI and high polygenic risk for Alzheimer's disease had reduced cortical thickness in Alzheimer's disease-vulnerable regions ($P = 0.019$, corrected $P = 0.048$; Table 3 and Fig. 2)

and this explained significant incremental variance in cortical thickness. These results remained significant after removing the 11 female participants from the dataset. There was no evidence that the number of mild TBIs (i.e. repetitive mild TBI) moderated the association between polygenic risk for Alzheimer's disease and cortical thickness ($P > 0.75$).

To further explore the pattern of results, we examined the relationship of mild TBI and low/high polygenic risk score groups (based on median split), with the caveat that this approach may be inherently less powerful than modelling using the continuous scale. Correcting for all covariates, between group comparisons revealed that subjects at high genetic risk for Alzheimer's disease and mild TBI had the lowest average cortical thickness values. Supplementary Fig. 1 shows a bar plot of the mean cortical thickness values of each group. The difference was significant in comparison to the high Alzheimer's disease genetic risk with no TBI group ($P = 0.004$) and the low Alzheimer's disease genetic risk with mild TBI group ($P = 0.015$). The pairwise test was not significant in comparison to the low Alzheimer's disease genetic risk no-TBI group ($P = 0.138$). The low genetic risk/no mild TBI group, low genetic risk/mild TBI group, and the high genetic risk/no mild TBI group were not significantly different from each other (all pairwise P 's > 0.2). The high Alzheimer's disease genetic risk and mild TBI group had lower cortical thickness values in comparison to the other three combined ($P = 0.004$).

To examine whether PTSD moderated the relationship between genetic risk and cortical thinning, we entered the PTSD by Alzheimer's disease polygenic risk score interaction to the regression model. The main effect of PTSD, and the interaction with Alzheimer's disease polygenic risk were not significant (P 's > 0.3 , see Supplementary Table 2). Further, the interaction between mild TBI and PTSD, without including Alzheimer's disease polygenic risk in the equation, was not significant ($P > 0.5$). These results did not change after removing females from the analysis. Taken together, these results suggest that PTSD was not associated with cortical thickness in Alzheimer's disease-sensitive regions, either alone or in conjunction with mild TBI or polygenic risk for Alzheimer's disease.

Greater cortical thinning with time since mild TBI and high genetic risk

To examine evidence for a neurodegenerative process among those at high genetic risk for Alzheimer's disease, we computed the amount of time (years) that had elapsed since the last reported TBI and added the interaction of this time variable and polygenic risk to the model (limited to participants with TBI). The interaction effect between time of last mild TBI and polygenic risk for Alzheimer's disease was marginally significant when including both males and the seven females with mild TBI ($n = 105$, $P = 0.07$), but was significant when including males only ($n = 98$,

Table 3 Summary of regression analysis for association with cortical thickness in Alzheimer's disease-vulnerable regions

Variable	Model 1				Model 2				Model 3			
	B	SE(B)	β	P	B	SE(B)	β	P	B	SE(B)	β	P
Age	-0.005	0.001	-0.350	<0.001 ^{***}	-0.005	0.001	-0.350	<0.001 ^{***}	-0.005	0.001	-0.347	<0.001 ^{***}
Sex	0.018	0.038	0.035	0.641	0.018	0.038	0.037	0.624	0.028	0.037	0.056	0.454
WTAR	0.001	0.000	0.098	0.200	0.001	0.000	0.100	0.186	0.001	0.000	0.126	0.094
PSQI	-0.027	0.022	-0.095	0.232	-0.018	0.022	-0.066	0.404	-0.018	0.022	-0.064	0.413
Lifetime PTSD	-0.130	0.22	-0.047	0.557	-0.009	0.023	-0.033	0.694	-0.011	0.022	-0.039	0.629
Lifetime mild TBI					-0.035	0.021	-0.131	0.096	0.279	0.135	1.05	0.04 [*]
PRS-AD					-0.002	0.001	-0.146	0.052	0.002	0.002	0.116	0.384
mTBI × PRS-AD									-0.006	0.003	-1.21	0.019 ^{a,*}
R ²		0.146				0.182				0.212		
F for ΔR^2		5.246 [*]				3.425 [*]				5.579 [*]		

mTBI = mild traumatic brain injury; PRS-AD = polygenic risk score for Alzheimer's disease.

^aCorrected value of this interaction term = 0.048 at the PRS-AD threshold $P < 0.2$.

^{*} $P < 0.05$, ^{**} $P < 0.01$. SE=standard error.

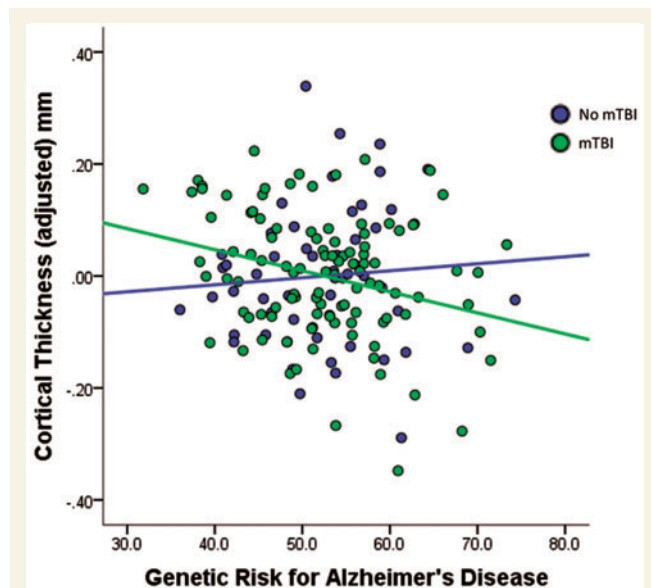


Figure 2 Reduced cortical thickness among subjects with mild TBI and high polygenic risk for Alzheimer's disease.

Values on the x-axis represent polygenic risk scores for Alzheimer's disease, with higher scores indicating increased risk for Alzheimer's disease. Values on the y-axis represent unstandardized residuals (i.e. the difference between predicted and observed cortical thickness values accounting for age, sex, PSQI, WTAR, and PTSD).

mTBI = mild traumatic brain injury.

$P = 0.042$, Fig. 3). This effect was age-independent (as age was accounted for in the model), and was not replicated when entering age at injury in place of time since injury ($P > 0.7$). Calculation of predicted values from the male-only regression model revealed that cortical thinning in individuals with high genetic risk (e.g. risk score = 95th percentile) was greater by 0.14 mm 10 years post-injury, 0.22 mm 20 years post-injury, and 0.31 mm 30 years post-injury than individuals with low genetic risk

(e.g. 10th percentile). With the caveat that these results are cross-sectional, they may suggest that cortical thinning increases over time, signalling a neurodegenerative process among those with high genetic risk for Alzheimer's disease.

Mild TBI does not moderate *APOE* or other Alzheimer's disease candidate single nucleotide polymorphisms

There was no main effect of *APOE* on cortical thickness, nor was there a mild TBI by *APOE* interaction. Further, when including polygenic risk for Alzheimer's disease scores in the model, the polygenic score was still associated with cortical thickness after adjusting for *APOE* status. These analyses did not change when females were removed. Linear models showed that only one SNP, rs4147929 in *ABCA7* showed a significant mild TBI × SNP effect on cortical thickness ($P = 0.043$). However, this effect did not survive multiple comparisons correction (Supplementary Table 3).

Association between mild TBI, polygenic risk for Alzheimer's disease, and individual Alzheimer's disease-vulnerable brain regions

We next explored whether mild TBI moderated the relationship between polygenic risk and any particular region of interest and hemisphere among the Alzheimer's disease-vulnerable regions examined. The strongest association for the interaction between mild TBI and polygenic risk was observed in the right posterior cingulate cortex (corrected for multiple comparisons, $P = 0.019$; see Table 4). The interaction was also associated with the left temporopolar cortex ($P = 0.021$), but this effect did not survive multiple comparisons correction (Supplementary Table 4). These results did not change when females were removed.

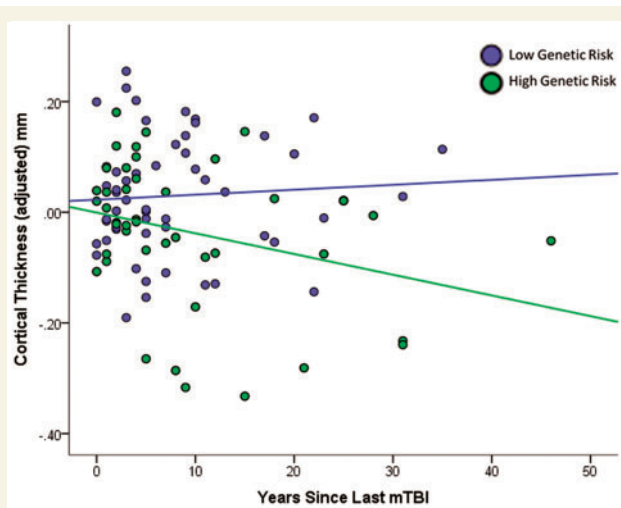


Figure 3 Greater reduction in cortical thickness is observed earlier post-injury among males with mild TBI and high polygenic risk for Alzheimer's disease. Time since injury (x-axis) = age at study – age at most recent mild TBI. Values on the y-axis represent unstandardized residuals (i.e. the difference between predicted and observed cortical thickness values accounting for age, PSQI, WTAR, and PTSD). Polygenic risk scores were dichotomized (median split) for illustration purposes only. Regression lines represent the best fit line for the low and high polygenic risk for Alzheimer's disease groups. mTBI = mild traumatic brain injury.

Specificity of Alzheimer's disease-vulnerable cortical regions and polygenic risk scores

We next examined mild TBI and genetic associations with seven control regions of interest. The mild TBI by polygenic risk score interaction did not significantly predict average cortical thickness in these control regions, suggesting specificity to the previously determined Alzheimer's disease-vulnerable brain regions (Supplementary Table 5). Further, to determine specificity of the polygenic risk score for Alzheimer's disease, we examined whether mild TBI moderated the relationship between polygenic risk for depression and schizophrenia and cortical thickness. There were no significant interactions between mild TBI and any *P*-value threshold for either the depression or schizophrenia polygenic risk scores in predicting cortical thickness.

Mild TBI and genetic risk for Alzheimer's disease influence delayed memory indirectly through cortical thickness

Finally, we examined whether the mild TBI by polygenic risk for Alzheimer's disease effect on cortical thickness had implications for memory performance. Delayed memory

tests are considered to be the most sensitive in detecting Alzheimer's disease in the early stages, thus we examined the long-delay free recall scores from the CVLT-II. First, we tested the hypothesis that polygenic effects confer risk for episodic memory loss through disturbances in cortical thickness, with mild TBI moderating the polygenic effect. The interactive effects of Alzheimer's disease polygenic risk by mild TBI interaction on cortical thickness and of cortical thickness to delayed memory were both significant adjusting for age, sex, PSQI, PTSD, and premorbid IQ. Further, a bias-corrected bootstrap confidence interval for the indirect effect of the highest order interaction (i.e. testing the hypothesis that the mild TBI \times polygenic risk interaction influences memory performance indirectly through cortical thickness) did not encompass zero [$ab = -0.026$, 95% confidence interval (CI) -0.08 to -0.002], suggesting a significant model. As expected, the combination of mild TBI and higher Alzheimer's disease polygenic risk scores was associated with reduced CVLT-II long-delay free recall scores through loss of cortical thickness in Alzheimer's disease-vulnerable brain regions (Fig. 4).

Discussion

This study examined the associations of mild TBI, polygenic risk for Alzheimer's disease, and their interaction with cortical thickness in brain regions susceptible in Alzheimer's disease. There were three main findings. First, the combination of mild TBI exposure and high polygenic risk for Alzheimer's disease was associated with reduced cortical thickness in Alzheimer's disease-vulnerable brain regions. Second, cortical thinning in Alzheimer's disease-vulnerable regions was accelerated post-injury (indexed by years elapsed since the last reported mild TBI) among males with high genetic risk for Alzheimer's disease. Finally, mild TBI and polygenic risk-related cortical thinning was associated with reduced delayed episodic memory recall.

Findings of this study shed new light on potential mechanisms of reduced cortical thickness following mild TBI by implicating genetic pathways associated with Alzheimer's disease including those involved in clathrin-mediated endocytosis signalling, inflammation, immune response, and cholesterol (Jones *et al.*, 2010; Logue *et al.*, 2014). Clathrin-mediated endocytosis has been associated with amyloid precursor protein (APP) processing and amyloid- β production (Wu and Yao, 2009). APP also plays a role in mild TBI and is considered a reliable neural marker of injury (Blumbergs *et al.*, 1995), suggesting that the clathrin-mediated pathway warrants additional study. Further, genetic pathway analyses in animal models of TBI have shown greater-than-chance involvement of genes associated with inflammation and the immune response (Crack *et al.*, 2009) and new data in humans suggest changes in gene expression related to markers of inflammation and immune response following concussion (Merchant-Borna *et al.*, 2016). The higher prevalence of

Table 4 Summary of regression analysis for association with cortical thickness in the right posterior cingulate cortex

Variable	Model 1				Model 2				Model 3			
	B	SE(B)	β	P	B	SE(B)	β	P	B	SE(B)	β	P
Age	−0.009	0.002	−0.301	<0.001 ^{***}	−0.009	0.002	−0.300	<0.001 ^{***}	−0.008	0.002	−0.292	<0.001 ^{***}
Sex	−0.050	0.074	−0.052	0.503	−0.048	0.075	−0.051	0.519	−0.022	0.073	−0.024	0.759
WTAR	0.000	0.001	−0.013	0.864	0.000	0.001	−0.011	0.888	0.000	0.001	0.027	0.730
PSQI	−0.003	0.043	−0.006	0.939	0.003	0.044	0.006	0.943	0.005	0.042	0.009	0.910
Lifetime PTSD	−0.039	0.043	−0.074	0.375	−0.037	0.045	−0.071	0.414	−0.042	0.044	−0.081	0.338
Lifetime mild TBI					−0.024	0.042	−0.048	0.559	0.834	0.263	1.651	0.002 ^{***}
PRS-AD					−0.002	0.002	−0.072	0.358	0.009	0.004	0.306	0.027 [*]
Mild TBI × PRS-AD									−0.016	0.005	−1.751	0.001 ^{a,***}
R ²		0.095				0.102				0.163		
F for ΔR^2		3.227 [*]				0.613				10.94 ^{**}		

PRS-AD = polygenic risk score for Alzheimer's disease.

^aCorrected value of this interaction term = 0.019 at the PRS-AD threshold $P < 0.2$.

* $P < 0.05$, ** $P < 0.01$.

stroke, diabetes, hyperlipidaemia, hypertension, and coronary artery disease previously reported in patients with TBI versus controls (Wang *et al.*, 2012) points to the potential involvement of gene pathways involved in cholesterol and atherosclerosis. Future research should examine these and other mechanisms by which TBI may lead to the onset of reduced cortical thickness.

There is growing evidence from animal and human work suggesting accelerated loss of structural brain integrity following TBI (Trotter *et al.*, 2015). A recent study showed premature brain atrophy in grey and white matter in individuals with TBI (Cole *et al.*, 2015). In this study, premature brain atrophy increased with passage of time since injury, similar to the results reported here, which are suggestive of cortical thinning as a function of the time since injury. In addition, both the data reported in Cole *et al.* (2015) and our data revealed that smaller brain structure estimates were associated with worse cognitive performance. Of note, Cole and colleagues (2015) found accelerated brain atrophy only in moderate and severe TBI cases, but not mild TBI, consistent with the notion that severity of injury is an important factor in predicting neurodegeneration. It is possible that for individuals with mild TBI, the biomechanical force of the injury is insufficient to elicit neurodegenerative processes unless also accompanied by additional risk, such as a predisposing genetic risk for Alzheimer's disease as reported here.

The primary brain region associated with the interaction of mild TBI and genetic risk for Alzheimer's disease was the posterior cingulate cortex. Previous studies have found early structural degeneration (Lehmann *et al.*, 2010), amyloid deposition (Buckner *et al.*, 2005), and altered functional energy metabolism in this region in Alzheimer's disease (Minoshima *et al.*, 1994). In addition, reduced perfusion in the posterior cingulate has been observed in individuals with mild cognitive impairment who go on to develop Alzheimer's disease (Johnson *et al.*, 1998). This region was also identified by Sabuncu and colleagues

(2012) as the area most strongly associated with their Alzheimer's disease polygenic score. Importantly, a recent study showed that white matter volume of the posterior cingulate/isthmus reduced over time following mild TBI (Zhou *et al.*, 2013). Taken together, these results suggest that the posterior cingulate may be vulnerable to both degeneration secondary to mild TBI and genetic risk for Alzheimer's disease, making it particularly susceptible to accelerated reductions in cortical thickness with the combination of TBI and neurodegenerative disease risk. We observed hemispheric asymmetry, with the greatest cortical thinning in the right posterior cingulate cortex. Although this finding was not hypothesized, there is evidence for the right posterior cingulate cortex as a strong marker for distinguishing stable versus progressive brain atrophy (Querbes *et al.*, 2009). Additional work is required to understand the role of hemispheric asymmetry in the present results.

Results of this study also showed that cortical thickness in Alzheimer's disease-vulnerable brain regions was associated with reduced performance on the CVLT-II long-delay free recall task, a sensitive measure of Alzheimer's disease-associated cognitive impairment. The CVLT-II long-delay task requires the participant to recall items from a learned list of 16 words after a 20-min delay. Tests that measure forgetting over a delay of minutes have been shown to differentiate patients with Alzheimer's disease in the early stages of the disease from healthy elderly controls with 85–90% accuracy (Knopman and Ryberg, 1989; Welsh *et al.*, 1991). In the present study, there was no direct effect of mild TBI on performance of this task. However, when genetic risk for Alzheimer's disease and cortical thinning in Alzheimer's disease-vulnerable regions were taken into account, there was a clear relationship between these variables and episodic memory performance, suggesting that only those individuals with mild TBI who also have early neurodegenerative markers show early changes in cognition. The results also

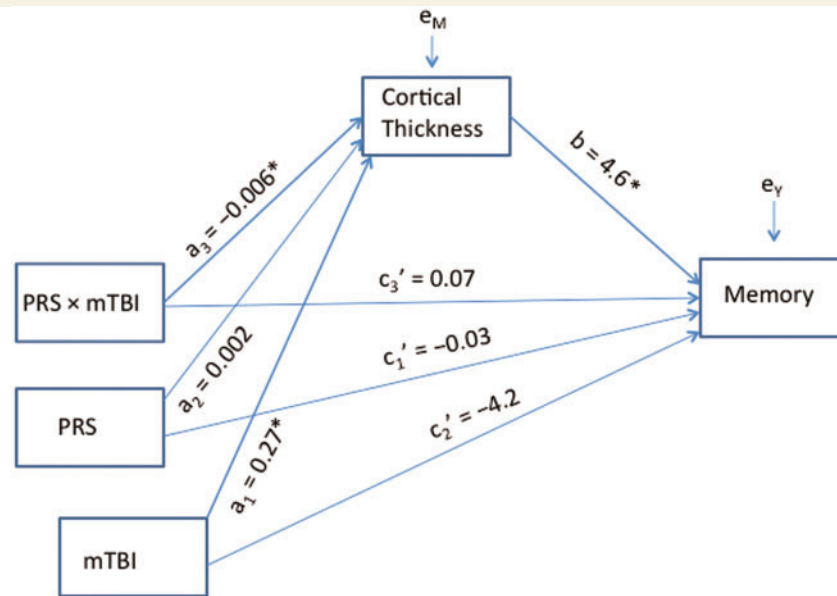


Figure 4 Moderated mediation model. Mild TBI moderated the relationship between polygenic risk for Alzheimer's disease and cortical thickness, and indirectly influenced delayed episodic memory performance. Covariates (age, sex, lifetime PTSD, PSQI, WTAR estimated IQ) were included in the model but are not shown here for display simplicity. PRS = polygenic risk for Alzheimer's disease score; mTBI = mild traumatic brain injury.

suggest that cortical thickness may be an important and sensitive indicator of early neurodegeneration and align with those of Sabuncu and colleagues (2012), who also found a relationship between genetic risk for Alzheimer's disease and cortical thickness. By contrast, studies that have used cognitive performance as a measure of early neurodegenerative risk without considering brain metrics have not found positive associations between TBI, genetic risk and neuropsychological task performance (e.g. Millar *et al.*, 2003; Rapoport *et al.*, 2008).

Although *APOE* represents one of the strongest risk factors for Alzheimer's disease, *APOE* or other single loci did not interact with mild TBI to predict cortical thickness. This result further underscores the power of a genome-wide polygenic approach to studying complex phenotypes and is consistent with other data showing that single candidate genes such as *APOE* explain significantly less of the phenotypic variance in Alzheimer's disease than polygenic scores (Ridge *et al.*, 2013). PTSD was also not associated with cortical thickness in Alzheimer's disease-related regions either directly or in combination with mild TBI or polygenic risk for Alzheimer's disease, raising the possibility that mild TBI has a distinct neural signature from PTSD. Indeed, recent studies have shown that the neurobehavioral outcomes seen in mild TBI, despite overlapping with PTSD, may be mediated by separate neural processes (Miller *et al.*, 2016). One implication of these findings is that documentation and assessment of mild TBI separately from PTSD is important, despite the sometimes near impossibility of attributing behavioural outcomes to one diagnosis over another. This is because although the behavioural

manifestation of these two diagnoses may be indistinguishable closer to the time of the injury, the long term outcomes may ultimately be different when they are combined with additional risk factors such as polygenic risk for neurodegenerative disease.

Repetitive mild TBI did not moderate the relationship between polygenic risk for Alzheimer's disease and cortical thickness. One possibility for the negative finding is that repetitive mild TBI may moderate genetic risk for neural disruptions in regions more consistent with chronic traumatic encephalopathy pathology, including the depths of the sulci preferentially in temporal and frontal lobes (McKee *et al.*, 2013) rather than regions related to Alzheimer's disease pathology as examined here. Another possibility is that the repetitive mild TBIs occurred far enough apart in the present sample to allow for recovery. Animal studies typically have multiple exposures within a predetermined period, and thus there may be a critical time window in following the first TBI within the second insult must occur to observe increased severity of brain injury over a single mild TBI. Additional studies are necessary to examine whether either of these possibilities are supported.

This study has several limitations that should be taken into account. First, although we observed reduced cortical thickness in regions that were shown previously to be associated with Alzheimer's disease, we cannot determine whether any of the individuals in this study will develop Alzheimer's disease or another neurodegenerative disease in the future, or whether lower thickness was present prior to injury. For example, we cannot rule out the possibility that

individuals with thinner cortices in Alzheimer's disease-related regions were more likely to sustain mild TBI. Thus, we are currently following the subjects in this study longitudinally to determine the association between cortical thinning and neurodegenerative disease. Longitudinal data will also help definitively determine whether cortical thinning is accelerated over time for a given subject, which could not be determined here given the cross-sectional nature of this study. Second, although we used a polygenic risk score for Alzheimer's disease, it is yet unknown whether the risk score is also associated with chronic traumatic encephalopathy or other dementias. Currently, the research on chronic traumatic encephalopathy is in its infancy and there are no GWASs of chronic traumatic encephalopathy. Given that the neuropathology of chronic traumatic encephalopathy has shared features with Alzheimer's disease including tau and amyloid deposits, at this stage it is impossible to determine whether these subjects would likely develop Alzheimer's disease versus another neurodegenerative disease. However, the finding that cortical thickness in Alzheimer's disease-vulnerable regions was associated with a cognitive domain most tied to Alzheimer's disease over other dementias (i.e. delayed recall), and mediated the relationship between mild TBI and polygenic risk and episodic memory provides support for the polygenic risk as a measure of Alzheimer's disease risk. Self-reported mild TBI is another limitation of the current study. We did not have access to corroborating military medical records, which for many military personnel, were often lost or destroyed because of environmental conditions in the field (Benson *et al.*, 2013). However, given that this is a limitation for most other studies of this nature, semi-structured interviews are the acknowledged standard for diagnosing remote mild TBI. Further, disentangling symptoms of psychological trauma or the sequelae of physical injury due to mild TBI in this sample is a challenge and adds to the uncertainty of mild TBI diagnosis. Another limitation is the selectivity of the sample. While representative of veterans of the recent wars, the low number of females in the study precluded us from having enough power to detect potential gender differences. In addition, these findings may not generalize to ethnic groups other than Caucasian, non-Hispanic individuals or non-military samples. Additional work is necessary to determine how cortical thinning rates in military samples compare to civilian TBI and neurological samples. Finally, we note that the findings from this study should be considered provisional until replication from additional samples is obtained.

In conclusion, we report here that the combination of mild TBI exposure and high genetic risk for Alzheimer's disease is associated with reduced cortical thickness in brain regions sensitive to neurodegeneration and is accompanied by reduced delayed memory recall performance. These results underscore the importance of studying the long term consequences of mild TBI in the large numbers of Iraq and Afghanistan service members returning with

exposure to mild TBI because of the potential public health ramifications for long-term care.

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Supplementary material

Supplementary material is available at *Brain* online.

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