Advanced Brain Age in Deployment-Related Traumatic Brain Injury: A LIMBIC-CENC Neuroimaging Study

Emily L Dennis[1,2], Brian A Taylor[3], Mary R Newsome[4,5], Maya Troyanskaya[4,5], Tracy J Abildskov[1], Aaron M Betts[6,7], Erin D Bigler[1,8,9], James Cole[10,11], Nicholas Davenport[12,13], Timothy Duncan[14], Jessica Gill[15,16], Vivian Guedes[15], Sidney R Hinds II[17], Elizabeth S Hovenden[1], Kimbra Kenney[17,18], Mary Jo Pugh[19,20], Randall S Scheibel[4,5], Pashtun-Poh Shahim[21], Robert Shih[7], William C Walker[22,23], Kent Werner[17], Gerald E York[24], David X Cifu[22], David F Tate[1,2], Elisabeth A Wilde[1,2,5]

1 Department of Neurology, University of Utah School of Medicine, Salt Lake City, UT.
2 George E. Wahlen Veterans Affairs Medical Center, Salt Lake City, UT.
3 Department of Imaging Physics, The University of Texas M. D. Anderson Cancer Center, Houston, TX.
4 Michael E. DeBakey Veterans Affairs Medical Center, Houston, TX.
5 H. Ben Taub Department of Physical Medicine and Rehabilitation, Baylor College of Medicine, Houston, TX.
6 Brooke Army Medical Center, Fort Sam Houston, TX.
7 Department of Radiology and Radiological Sciences, Uniformed Services University, Bethesda, MD.
8 Department of Psychology, Brigham Young University, Provo, UT.
9 Neuroscience Center, Brigham Young University, Provo, UT.
10 Dementia Research Centre, Institute of Neurology, University College London, London, UK.
11 Centre for Medical Image Computing, Department of Computer Science, University College London, London, UK.
12 Minneapolis VA Health Care System, Minneapolis, MN.
13 Department of Psychiatry and Behavioral Sciences, University of Minnesota Medical School, Minneapolis, MN.
14 VA Portland Healthcare Center, Portland, OR.
15 National Institutes of Health, National Institute of Nursing Research, Bethesda, MD.
16 Center for Neuroscience and Regenerative Medicine (CNRM), Uniformed Services University, Bethesda, MD.
17 Department of Neurology, Uniformed Services University, Bethesda, MD.
18 National Intrepid Center of Excellence, Walter Reed National Military Medical Center, Bethesda, MD.
19 Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT.
20 Information Decision-Enhancement and Analytic Sciences Center, VA Salt Lake City, Salt Lake City, UT.
21 Rehabilitation Medicine Department, National Institutes of Health Clinical Center, Bethesda, MD.
22 Department of Physical Medicine and Rehabilitation, Virginia Commonwealth University, Richmond, VA.
23 Hunter Holmes McGuire Veterans Affairs Medical Center, Richmond, VA.
24 Alaska Radiology Associates, Anchorage, AK.

Abstract

Objective: To determine if history of mild traumatic brain injury (mTBI) is associated with advanced or accelerated brain aging among United States (US) military Service Members and Veterans.

Methods: 822 participants (mean age=40.4 years, 714 male/108 female) underwent MRI sessions at eight sites across the US. 201 participants completed a follow-up scan between five months and four years later. Predicted brain ages were calculated using T1-weighted MRIs and then compared with chronological ages to generate an Age Deviation Score for cross-sectional analyses and an Interval Deviation Score for longitudinal analyses. Participants also completed a neuropsychological battery, including measures of both cognitive functioning and psychological health.

Result: In cross-sectional analyses, males with a history of deployment-related mTBI showed advanced brain age compared to those without ($t(884)=2.1$, $p=0.038$), while this association was not significant in females. In follow-up analyses of the male participants, severity of posttraumatic stress disorder (PTSD), depression symptoms, and alcohol misuse were also associated with advanced brain age.

Conclusion: History of deployment-related mTBI, severity of PTSD and depression symptoms, and alcohol misuse are associated with advanced brain aging in male US military Service Members and Veterans.

Keywords: neuroimaging, TBI, mild brain injury, aging, MRI

Introduction

Mild traumatic brain injury (mTBI) has been called one of the “signature injuries” of the recent conflicts in Iraq and Afghanistan, particularly when due to blast exposure (1). Such injuries, the majority of which are mild, appear more prevalent than in prior conflicts due to differences in both the combat environment and improved screening measures, as well as the length of the conflict (2). Neuroimaging methods show promise as biomarkers of injury and recovery; however, small samples often limit reproducibility and generalizability. Additionally, outcome after injury is heterogeneous and not fully explained by injury severity, indicating that pre- and/or post-injury factors also affect outcome. Advances in imaging methods and the collection of coordinated multi-site data hold great promise in elucidating factors that influence recovery (3).

Although acute symptoms of mTBI typically resolve within weeks, some patients may experience long-term sequelae. Increasing evidence indicates that TBI is not an isolated life event, but may have future implications regarding brain health and may correlate with neurodegeneration. A history of TBI is associated with an increased risk of early cognitive decline (4) and general repetitive head trauma may lead to the development of age-related neuropathologies (5). While both civilian and military cohort prospective studies with long-term follow-up (25-50 years post-injury) suggest that moderate/severe TBI (6, 7) or TBI with chronic deficit or dysfunction (8), may contribute to risk of developing dementia years after injury, literature regarding dementia risk associated with remote mTBI is less established (9). There is a growing literature regarding sex differences in post-injury outcome, with most studies suggesting poorer outcome in females, however there is no consensus regarding this effect (10, 11).

In studies of dementia or general cognitive decline, it is important to characterize and distinguish the effects of normal versus pathologic brain aging. Characteristics of typical brain aging include volume loss throughout the brain, with the rate of decline varying between brain regions (12–15), thinning of the cortical ribbon, with loss in frontal areas occurring before declines in posterior regions (16), and reduced segregation in network connectivity (both structural and functional) (17). These processes appear to differ between males and females, as a number of studies have shown evidence of accelerated decline in males relative to females (18–20). A number of approaches exist to estimate a “brain age” through comparison of an individual’s brain scan to a model generated from the data of hundreds or thousands of healthy participants (21). Studies of moderate/severe TBI show accelerated aging in brain volume (22, 23), while individuals with military-related

mTBI (24) evidenced advanced brain age in terms of cortical thinning (24). Additionally, a history of mTBI may exaggerate aging of white matter (25); however, another study that measured white matter in Veterans with mTBI revealed an interaction between blast exposure and age, while an interaction between mTBI and age was nonsignificant (26). In the general population, studies show that older brain age, relative to chronological age, is associated with increased mortality, reduced cognitive function, and increased risk of dementia (27–29). This finding is consistent with evidence from a longitudinal study that reported mTBI-related neurobehavioral and psychiatric disruptions increase over time (30).

Further research using larger studies is needed to validate these findings and more thoroughly characterize the underlying structural and functional consequences following mTBI. This information may allow for identification of novel therapeutic targets for intervention - a major gap in clinical considerations regarding mTBI. In this investigation, we used structural MRI data to examine brain aging in a large, longitudinal sample by leveraging the Long-term Impact of Military-relevant Brain Injury Consortium-Chronic Effects of Neurotrauma Consortium (LIMBIC-CENC) Prospective Longitudinal Study to examine the association between mTBI history and brain aging in US military Service Members and Veterans (SMVs). We hypothesized that we would see evidence of both advanced brain aging (older brain ages in cross-sectional analyses) and accelerated brain aging (faster brain aging in longitudinal analyses) in individuals with a history of mTBI. Given the growing literature indicating significant sex-related differences in the injury and recovery processes and the established literature about sex differences in aging we hypothesized that we would see sex differences, but we did not hypothesize a direction because while age-related declines appear to be accelerated in males, most existing literature shows poorer outcomes in females after mTBI. Links between combat deployments and brain function increase understanding of how deployment-related injuries influence SMVs health, elucidate necessary support over the lifespan, and determine pathomechanisms that may be addressed for future therapeutic development.

Materials and Methods

Participants

Participants were included from a secondary analysis of data collected as part of the ongoing LIMBIC-CENC Prospective Longitudinal Study, which is described at length in prior publications (31–33). Briefly, the parent study collects longitudinal physical, cognitive, mental health, everyday functioning, demographic, fluid

biomarker, and neuroimaging data on post-9/11 era SMVs with a history of deployment and either positive or negative mTBI histories at eleven sites throughout the United States. At the time of this dataset extraction, eight sites actively participated in data collection. Institutional Review Boards at each site approved this study, and all participants signed an informed consent document prior to undergoing baseline assessment. Inclusion criteria were: 1) deployment in Operation Enduring Freedom (OEF), Operation Iraqi Freedom (OIF), Operation New Dawn (OND), or follow-on conflicts, 2) history of combat exposure defined by the Deployment Risk and Resiliency Inventory Section D (DRRI-2-D) score >1 on any item, and 3) at least 18 years of age. Exclusionary criteria were: 1) any history of moderate/severe TBI as defined by either a) Glasgow Coma Scale >13, b) coma duration >0.5 hour, c) post-traumatic amnesia (PTA) duration >24 hours, or d) traumatic intracranial lesion; or 2) history of major neurologic disorder with significant decrease in functional status and/or loss of ability for independent living (e.g., dementia) or severe psychiatric disorder (e.g., schizophrenia).

Table 1 reports the demographic data of 822 participants that provided usable data at one or two timepoints (explained below), including 108 females and 714 males (average age=40.4, SD=10.1 years). Of the 201 participants with usable longitudinal data, the average interval between scans was 1.6 years (SD=0.79, range=5 months - 4 years).

Clinical, Neuropsychological, and Psychiatric Data Collection

Using a structured interview, lifetime history of all possible concussive events (PCEs) was assessed (34). Every PCE was classified as mTBI versus not mTBI, during deployment or outside deployment, and as blast-related or non-blast, based on the mechanism of injury. Exposure to controlled and uncontrolled blast was also collected. Only individuals reporting symptoms consistent with a history of mTBI and those without lifetime TBI enrolled in this study; individuals with a history of moderate/severe TBI were excluded.

Effort and Symptom Validity Screening. The recommended cut-off scores on the Medical Symptom Validity Test and Neurobehavioral Symptom Inventory Validity-10 index were used to detect suboptimal effort on neuropsychological testing and symptom over-reporting, respectively (35, 36). *Substance Misuse.* Ongoing alcohol consumption and drug misuse were evaluated using the Alcohol Use Disorders Identification Test-Concise (AUDIT-C) and Drug Abuse Screening Test (DAST) (37, 38). *Emotional Functioning*. Assessment of current level of posttraumatic stress disorder (PTSD) symptom severity was obtained using The PTSD Checklist

for DSM-5 (PCL-5) and depressive symptoms assessed using the Patient Health Questionnaire-9 (PHQ-9) (39, 40). *Cognitive Functioning.* Visual memory and learning were evaluated using the Brief Visuospatial Memory Test-Revised (BVMT-R) Total Recall and Delayed Recall, and verbal memory and learning was assessed using the California Verbal Learning Test-II (CVLT-II) Total, Short, and Long Delay Free Recall scores (41, 42). Verbal fluency was evaluated using the Delis–Kaplan Executive Function System (D-KEFS) Letter Fluency and Category Switching Total correct variables (43). Processing speed was measured using the Trail-Making Test (TMT) (B-A) completion time and the Wechsler Adult Intelligence Scale 4th Edition (WAIS-IV) Processing Speed Index (PSI), and working memory using the WAIS-IV Digit Span subtest (44, 45). Raw scores were used for all cognitive measures as age and sex were included as covariates in analyses. All neuropsychological assessments were completed around the same time as the MRI scan, both at baseline and at the follow-up timepoint.

MRI acquisition

3D T1-W images were collected using a protocol recommended by the Alzheimer's Disease Neuroimaging Initiative for volumetric measurement (46). All sites implemented monitoring throughout the image acquisition period to ensure both quality and consistency (i.e., geometric accuracy, signal to noise, etc.). The scan parameters for the T1-weighted sequences at each of the eight sites are shown in **Supplementary Table 1**.

Brain Age Prediction

Raw T1-weighted MR images were processed with the brainageR workflow (https://github.com/james-cole/brainageR) with a model similar to those described previously (21, 22, 27). The brainageR model was trained on 3,377 individuals from seven publicly available datasets including individuals 18-100 years old from samples in the US, United Kingdom, Australia, and China. Briefly, T1-weighted images were segmented into gray and white matter and cerebral spinal fluid using SPM12 (47) and spatially normalized. The resulting images were vectorized and principal components analysis (using R *prcomp* https://cran.r-project.org) was run with the components explaining the top 80% of variance retained, resulting in 435 components. Processing for the LIMBIC-CENC data was the same, with raw T1-weighted images segmented, normalized, vectorized, and the

rotation matrix from the training dataset applied to yield 435 components for each participant. The resulting components were used to predict brain age using kernlab (48), and tissue segmentations were visually checked for quality.

Statistical Analyses

Of the 1407 scans across all eight LIMBIC-CENC sites with T1-weighted data collected, 15.6% failed visual quality control (QC). However, these primarily came from two sites with significantly higher fail rates than the others (63% and 36%). Of the remaining 6 sites, 2.5% failed visual QC, for a sample size of 1,038 scans. Due to missing demographic or clinical information for some participants, the final sample size was 822 total participants, 201 of whom were scanned twice, for a total of 1023 scans. In order to include the maximum amount of data, cross-sectional analyses included all available scans that passed QC.

In cross-sectional analyses, the variable of interest was Age Deviation Score (ADS), calculated by subtracting the chronological age from the predicted age. For longitudinal analyses, the variable of interest was Interval Deviation Score (IDS), calculated by subtracting the chronological interval from the predicted interval. In both cases, a negative score is “good”, indicating a younger brain age or slower calculated aging interval. Plots of the chronological age and predicted brain age across sites are shown in **Figure 1**. Statistical analyses were run as mixed effects models in R 3.1.3 with the *nlme* package with ADS or IDS as the dependent variable. Nested random effects (intercept) were used to control for site and participant. A significant correlation between age and ADS (r=-0.33, p=1.6x10^{-27}) and a significant sex effect on ADS (t(1021)=-5.8, p=1.2x10^{-8}) were demonstrated; thus, both age and sex were included as covariates in all analyses (with the exception of those separated by sex).

Primary Analyses - Group and Interactions

Our primary analyses focused on group differences in ADS and IDS based on a history of deployment-related mTBI, blast-related mTBI, or any mTBI, as well as interactions between TBI history and sex. In the primary deployment-related and blast-related mTBI analyses, we included some individuals with non-deployment-related injuries in the “control” group, with follow-up analyses excluding these individuals for a “cleaner” control group.

Secondary Analyses - Clinical and Cognitive Variables

The clinical variables included both injury details and comorbidities, including history of blast mTBI, history of non-deployment mTBI, number of TBIs, number of PCEs, time since first, worst, and most recent TBI, and total scores for PCL-5, PHQ-9, DAST-10, and AUDIT-C. As described above, the scores of Validity-10 used for symptom validity screening resulted in the removal of data for 27 participants with results higher than the established cut-off score. As described above, the MSVT was used for performance validity screening, resulting in 53 participants being removed. **Figure 2** presents a flowchart describing the number of data points included versus excluded at each step.

Results

Primary Cross-sectional Analysis - Group

Across the full sample, we did not detect significant differences in ADS between groups based on history of deployment-related mTBI (p=0.25), blast-related mTBI (p=0.34), or any mTBI (p=0.21). We repeated the deployment and blast analyses comparing individuals to those with no history of mTBI (vs. a control group that may have a history of non-combat mTBI), and discovered similar results.

Primary Cross-sectional Analysis - Group by Sex Interaction

Across the full sample, we found an interaction between sex and history of deployment-related mTBI (t(1021)=-3.0, p=0.0028, **Figure 3**). A positive association between ADS and mTBI history in males was seen, while the association appeared negative in females (males: t(884)=2.1, p=0.038; females: t(135)=-2.4, p=0.017).

The association in males followed the expected direction, with a history of deployment-related mTBI associated with an increase in brain age of 0.95 years (95% CI: 0.035,1.86). In females, however, the association appeared in the opposite direction. To understand these findings, we examined both the group breakdown across sites and the composition of the controls. We discovered that three sites had extremely uneven groups, with fewer than five female participants reporting a history of deployment-related mTBI at each site (**Supplemental Figure 1**). Further, the control group included individuals with no history of mTBI along with those with a history of non-deployment mTBI. Examining the descriptions of each PCE, data for those individuals with non-

deployment mTBI revealed multiple instances of mTBI due to domestic violence such as intimate partner violence and/or child abuse, whereas the deployment and blast mTBI groups reported fewer of these events (although the comparison was not significant, deployment-related 1/40 vs. non-deployment related 5/68). A number of issues exist when including domestic violence TBI in the "control" group, including the repetitive nature of the exposure, frequency of comorbidities, and potential for hypoxic or anoxic events due to strangulation (49). When the analysis was rerun with only sites including five or more females in the mTBI group (Sites 1, 2, and 4), and comparing deployment-related mTBI to no history of TBI, the comparison was no longer significant ($t(63)=-1.3$, $p=0.19$).

Primary Longitudinal Analyses

Across the full longitudinal sample (N=201, 172M/29F), we did not find any significant associations between IDS and TBI history. Additionally, there were no significant interactions between TBI history and age or sex. Examining males and females separately, no significant associations in males were observed, but an association in females with the history of non-deployment mTBI was seen($t(27)=2.8$, $p=0.012$).

Secondary Analyses - Clinical and Cognitive Variables

Due to inconsistencies in the female sample and small sample size at several study sites discussed above, secondary analyses were completed in the male group only. History of blast mTBI was not significantly associated with ADS when compared to all controls (those with no TBI as well as non-blast mTBI) ($t(884)=1.6$, $p=0.11$); however, there was a significant difference in ADS between blast mTBI and no TBI ($t(487)=2.1$, $p=0.038$). We did not find an association between history of non-deployment mTBI and ADS ($t(884)=0.54$, $p=0.59$). Further, time since first, worst, and most recent TBI were not associated with ADS (all $p>0.4$). Number of blast PCEs and mTBIs were not associated with ADS (both $p>0.10$). Although the number of deployment-related PCEs was not associated with ADS, the number of deployment-related mTBIs showed a weak association ($t(884)=1.8$, $p=0.073$). PCL-5 total score was associated with ADS ($t(849)=2.7$, $p=0.0067$), as was PHQ-9 total score ($t(848)=3.1$, $p=0.0026$), and AUDIT-C total score ($t(877)=3.0$, $p=0.0030$) (**Figure 4**). For every one point increase in PCL-5 score, brain age was 1.5 weeks older; for every one point increase in PHQ-9 score, brain age was five weeks older; for every one point increase in AUDIT-C, brain age was eleven weeks older.

Among cognitive variables tested, an association with BVMT-R Delayed Recall raw score ($t(687)=2.0$, $p=0.045$) and a weak association with BVMT-R immediate recall raw score ($t(687)=1.8$, $p=0.079$) was observed. After correcting for multiple comparisons across the cognitive and psychological variables, only the associations with PCL-5, PHQ-9, and AUDIT-C remained significant (**Table 2**). Due to the considerable overlap between mTBI history, PCL-5, PHQ-9, and AUDIT-C severity, we also ran a partial *F*-test to determine whether the associations with mTBI were entirely due to one of the other measures. The full model included age, mTBI history, PCL-5, PHQ-9, and AUDIT-C scores. The reduced models included age plus any individual variable, or any combination of the above variables. In all cases, the full model explained significantly more variance in ADS than the reduced models (*F*-stats>5.3), indicating that each variable is contributing some unique information in explaining ADS.

Discussion

We present results from the LIMBIC-CENC Prospective Longitudinal Study showing evidence of advanced brain age following deployment-related mTBI in male SMVs. This association was present for deployment-related and blast-related mTBI, but not for non-deployment TBI, indicating that deployment-related injuries may be of particular concern for brain health. A history of deployment-related mTBI was associated with an increase in brain age of 0.95 years. This adds to the existing literature indicating that mTBI can have long-lasting implications for brain health, including advancing age-related neurodegeneration (24, 50, 51). Military-relevant mTBI can occur through a number of mechanisms, including blunt force trauma, blast exposure, or the combination of the two. Both the acceleration/deceleration and rotational forces of blunt trauma and the traveling pressure wave of blast TBI can stretch and shear axons, rupture blood vessels, and cause ionic imbalances, all of which can lead to a range of secondary injuries. Chronic inflammatory response, cerebrovascular disruption, oxidative stress, cellular dysfunction, and progranulin deficiency have also been implicated as mechanisms influencing subsequent neurodegeneration, though the degree to which these factors contribute to progessive pathological change has not been well established (52, 53). While the severity of TBI in the participants assessed here consisted entirely of mTBI, nearly 70% of those with a history of TBI reported more than one mTBI, with numerous additional potentially subconcussive exposures. Therefore, the reason deployment-related mTBI was associated with brain age, while non-deployment related mTBI was not associated, could be due to multiple mechanisms of TBI, a greater number of mTBIs, and secondary effects of the deployment setting such as added

duress and stress when sustained (54). A history of repetitive concussive and subconcussive exposures is associated with accelerated cognitive decline (5), though the strength of this association and proposed mechanisms are debated (55, 56). Cole et al. (2015) (11) reported an increased brain age of 4.66 years in civilian patients with moderate/severe TBI, whereas we report an increase of 0.95 years in SMVs with mTBI. Future studies may reveal a continuum whereby increased severity is positively associated with increased brain age as well as whether magnitude of change along the continuum is typified differently in different neurodegenerative diseases.

In the cross-sectional analyses, we report an unexpected effect in females, with a history of deployment-related mTBI associated with *lower* brain age. Probing the participant-reported event-level data, there were numerous reports of mTBI related to domestic violence. These reports were primarily intimate partner violence but also inluded reports of likely child abuse. Additionally, these were more prevalent in the non-deployment mTBI group versus the deployment-related mTBI group. Most of the reports of intimate partner violence include strangulation, and studies have suggested that such hypoxic/anoxic events exacerbate brain trauma (57, 58). Removing three sites with small, uneven group distributions, and comparing female participants with a history of deployment-related mTBI to those with no history of TBI revealed no significant effect. While the longitudinal results were largely null, we did see an association between history of non-deployment mTBI and accelerated brain aging. This finding requires replication, as it results from a small sample (N=29), but adds to the cross-sectional results in suggesting that both deployment and non-deployment mTBI in the female SMV population are significant health issues that warrant further targeted study (59).

Our analysis included several common comorbidities of TBI, including PTSD, depression, and alcohol and substance misuse. Results indicated that PTSD and depression symptom severity and alcohol misuse were associated with advanced brain age. Some studies have shown modest effects of depression and PTSD on brain age, using similar methods to operationalize brain age (60, 61), but their results have been mixed (62). Multiple studies show that alcohol misuse correlates with advanced or accelerated brain aging (63–65). One likely mechanism by which these disorders may lead to advanced brain aging is inflammation. Changes in immune function naturally occur with age, and stressors, especially chronic stressors such as depression and PTSD, can interact with the immune system to exacerbate dysregulation and accelerate decline (66–70). In turn, chronic stress exposure leads to dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to

cascading events that result in increased systemic inflammation and neuronal atrophy (with hypertrophy in areas such as the amygdala) (68). Ethanol may be directly neurotoxic (71), and intoxication also leads to inflammation and inhibits neurogenesis acutely (72). As with other insults to the body, TBI results in a neuroinflammatory response that is necessary for recovery. However, some individuals develop chronic inflammation, particularly those with moderate/severe TBI or repetitive mTBI (5, 73). As comorbid disorders, we needed to determine whether our reported associations with mTBI history were in fact due to PTSD, depression, or alcohol misuse, although it is difficult to truly untangle these disorders that may emerge concurrently. Partial *F*-tests revealed that each of these variables is contributing complementary information in explaining the variance in ADS.

With significant effects in the cross-sectional analyses, but not the longitudinal analyses, our results support the hypothesis that deployment-related mTBI is associated with advanced brain aging in males, but they do not support our hypothesis of accelerated brain aging. There could be several reasons for the lack of longitudinal results. First, mTBI could truly cause a shift in brain aging without changing the slope of the trajectory of aging. Second, while 201 longitudinal datasets is not small, across six sites, we may have insufficient sample size to detect these effects. Third, the interval between scans was not long (average 1.6 years) and our sample was relatively young and healthy. It could be that the accelerated aging processes due to mTBI history do not appear until later in life, or require a longer interval to be detectable. As the LIMBIC-CENC study continues with additional follow-up sessions, we will continue to examine brain age to determine whether evidence for accelerated aging becomes apparent over longer follow-ups or as the participants age.

Injury prevention and development of therapeutic interventions which may attenuate or reverse brain age advancement caused by mTBI and improve outcomes are important areas of research. Therapies to combat neurodegeneration after mTBI include red/near-infrared light-emitting treatments, which aim to improve cell metabolism and show improvements in cognition and symptom reduction (74) are promising, but require additional investigation. Some studies that report improved symptoms after behavioral intervention likely entail brain changes, and clinical trials involving brain measures in other patient populations and in healthy adults may have some application. In patients with more severe brain injuries, virtual reality treatment was linked to brain volume increases that were related to improvements in motor function (75). A systematic review of the effect of meditation on the brains of people with neurodegenerative disease reported increased brain volumes (76). Another linked meditation to increases in frontal and hippocampal volume and functional connectivity, as well as

improvement in the functional connectivity between the amygdala and frontal lobe (77). Meditation has also been associated with improved white matter organization around the anterior cingulate cortex (78). Additionally, physical exercise and dance have been associated with increases in brain volume and improvements in white matter organization (79, 80). In response to several studies reporting improvements directly on brains, including those of people with neurodegeneration, it may be timely to investigate the role of these easily accessible therapies on brains of SMVs, both with and without TBI. Additionally, interventions to increase physical and mental activity as well as general overall and cardiovascular health are likely beneficial in the context of aging and mTBI.

To our knowledge, this is the first published analysis of brain age using volumetric data as a measure of disruption in normal aging in mTBI, and our cohort represents the largest prospective study of military-relevant participants to date. However, we note a few limitations of our study. The process for determining a brain age employed here does not specify the features leading to that determination or the mechanisms underlying the changes. Reducing brain disruptions to a single number obviously significantly reduces the overall context; however, it does provide an intuitive way to communicate the magnitude of the issue. Additionally, this process proves useful for a disorder as heterogeneous as TBI, in that different patterns of alterations may exist across individuals and each of these are considered in the determination of a brain age. Group-level analyses of specific brain regions may not detect abnormalities that are uncommon across a large portion of the patient group. Regardless, it may be useful in future to identify on an individual basis whether certain brain regions are weighted more heavily in the determination of brain age, as this information may be associated with concurrent or future disruptions. Another limitation is the relatively short length of follow-up, as it may not be long enough to identify trajectories. However, as previously mentioned, additional follow-up data collection is ongoing, and will be analyzed in future. The relatively small number of female participants, especially those reporting a history of deployment-related mTBI, is also a limitation as it hinders our ability to delve into the specific factors impacting female SMV brain health. Additionally, while the structured interview described above provided a comprehensive evaluation of a participant's lifetime history of all PCEs, it is possible that the incidents related to domestic violence and military sexual trauma were underreported, as survivors may be hesitant to disclose these events. Although we assessed the current levels of PTSD symptoms, depression symptoms, and substance use, we did not assess past history for these factors. Given that concurrent PTSD, depression, and alcohol use were

associated with brain age, it is likely that past history of these also influences brain age. Studies with more in depth characterization may be able to address this, and we additionally plan to examine this in future LIMBIC-CENC analyses when more repeated measures of these factors are available. Despite these limitations, we believe that this study furthers our understanding of the impacts of deployment-related mTBI on SMV health, may encourage new methods of brain protection, and will be useful in preparing to support SMVs across the lifespan.

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Disclosure Statement

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Figure and Table Legends

Table 1. Sample demographics. Demographics for each of the six sites included in analyses are listed, for both the cross-sectional (CSX) and longitudinal (long) datasets, along with totals. The sample size, M/F breakdown, and mean age at baseline (standard deviation) are listed. The percent of the sample endorsing a history of any TBI, deployment-related TBI, and blast TBI are listed.

Figure 1. Plots of chronological age versus predicted age across sites. The chronological age (x) is plotted against the predicted brain age (y) for each of the 6 sites. In black is the y=x line for reference.

Figure 2. Sample size flowchart. From the initial available cohort of 1,407 datapoints, participants were excluded at various steps for QC or data availability issues. The number included vs. excluded at each step are listed below. Nota bene - the N listed indicates the number of data points, not participants, as some participants contributed data at two timepoints. This was accounted for in the statistical models. AUDIT-C - Alcohol Use Disorders Identification Test; BVMT-R - Benton Visual Retention Test - Revised; CVLT-II - California Verbal Learning Test - Second Edition; DAST-10 - Drug Abuse Screening Test; D-KEFS - Delis Kaplan Executive Function System; PCL-5 - PTSD Checklist for DSM-5; PHQ-9 - Patient Health Questionnaire, 9-Item; TMT - Trail Making Test (Trial B - Trial A); WAIS-IV: Wechsler Adult Intelligence Test, Fourth Edition.

Figure 3. Interaction between sex and history of deployment-related TBI. Violin plots for the ADS (age deviation score) for the deployment-related TBI group (blue) and control group (pink) are shown, separated by sex.

Figure 4. Associations with PCL-5, PHQ-9, AUDIT-C. Total scores on the PCL-5 (PTSD Checklist for DSM-5), PHQ-9 (Patient Health Questionnaire), and AUDIT-C (Alcohol Use Disorders Identification Test) are plotted against ADS (age deviation score) with linear plot line in blue and 95% CI in grey. Red lines indicate generally accepted clinical cutoffs (PCL-5>32, PHQ-9>9, AUDIT-C>7).

Table 2. Cognitive and psychological variables (males only). Associations between brain age and cognitive and psychological health variables are shown, with *T*-statistics, raw *p*-values, and FDR corrected *p*-values. *Italics* denotes $p<0.05$ prior to correction for multiple comparisons, **bold** font denotes $p<0.05$ after correction for multiple comparisons. AUDIT-C - Alcohol Use Disorders Identification Test; BVMT-R - Benton Visual Retention Test - Revised; CVLT-II - California Verbal Learning Test - Second Edition; DAST-10 - Drug Abuse Screening Test; D-KEFS - Delis Kaplan Executive Function System; PCL-5 - PTSD Checklist for DSM-5; PHQ-9 - Patient Health Questionnaire, 9-Item; WAIS-IV: Wechsler Adult Intelligence Test, Fourth Edition.

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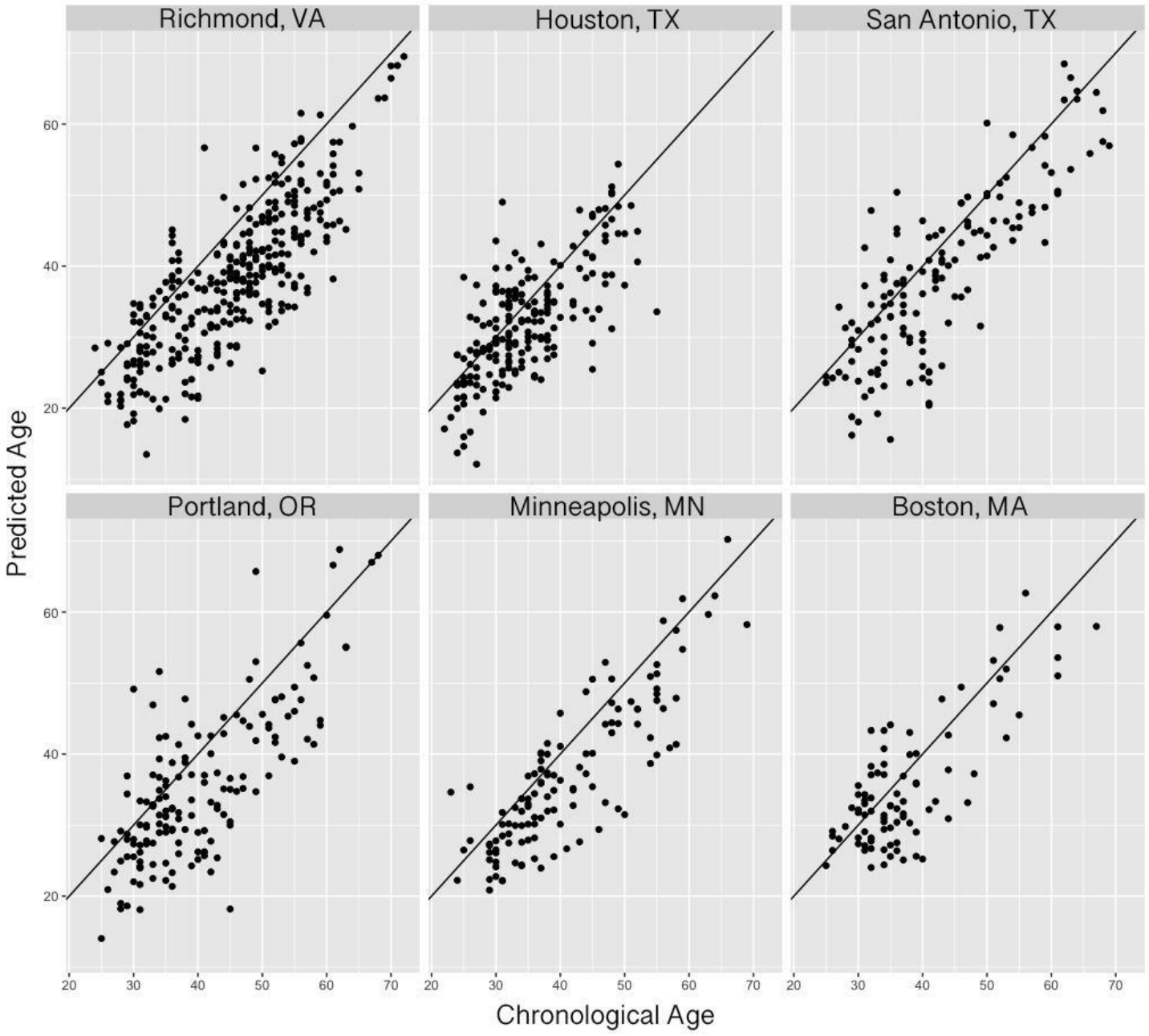
	Total CSX N (M/F)	Total long N (M/F)	Mean age at baseline (SD)	Any TBI	Deployment-related TBI	Blast TBI
Richmond, VA (Site 1)	252 (207/45)	73 (64/9)	44.6 (9.7)	77%	47%	31%
Houston, TX (Site 2)	163 (149/14)	37 (33/4)	34.2 (7.3)	82%	67%	44%
San Antonio, TX (Site 4)	120 (104/16)	21 (17/4)	42.2 (10.7)	89%	62%	46%
Portland, OR (Site 6)	116 (100/16)	36 (28/8)	40.0 (9.4)	84%	35%	22%
Minneapolis, MN (Site 7)	96 (86/10)	22 (19/3)	41.0 (10.2)	68%	34%	17%
Boston, MA (Site 8)	75 (68/7)	12 (11/1)	37.4 (9.1)	89%	64%	40%
Total	**822 (714/108)**	**201 (172/29)**	**40.4 (10.1)**	**81%**	**52%**	**34%**

Figure 1. Plots of chronological age versus predicted age across sites. The chronological age (x) is plotted against the predicted brain age (y) for each of the 6 sites. In black is the y=x line for reference.



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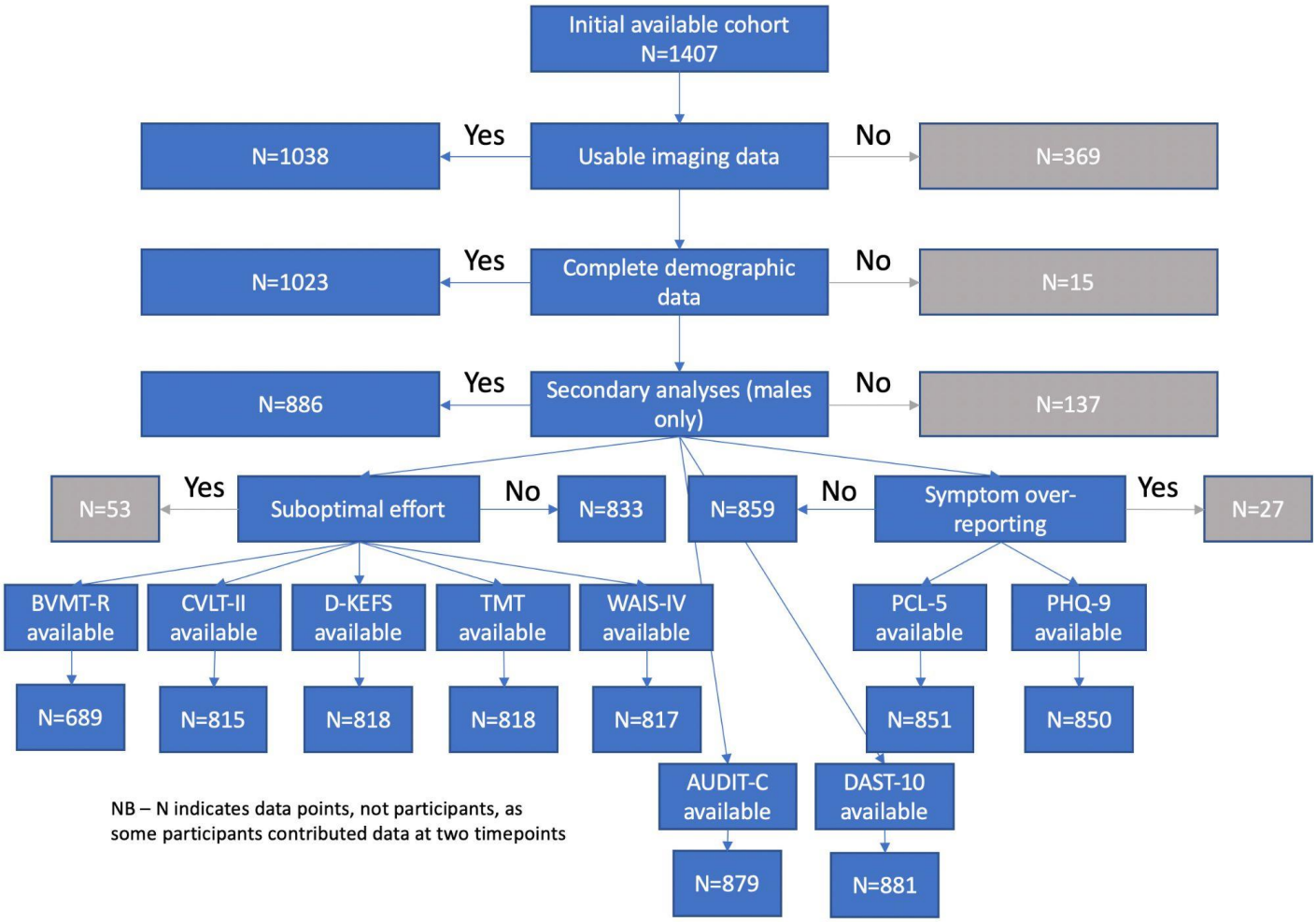

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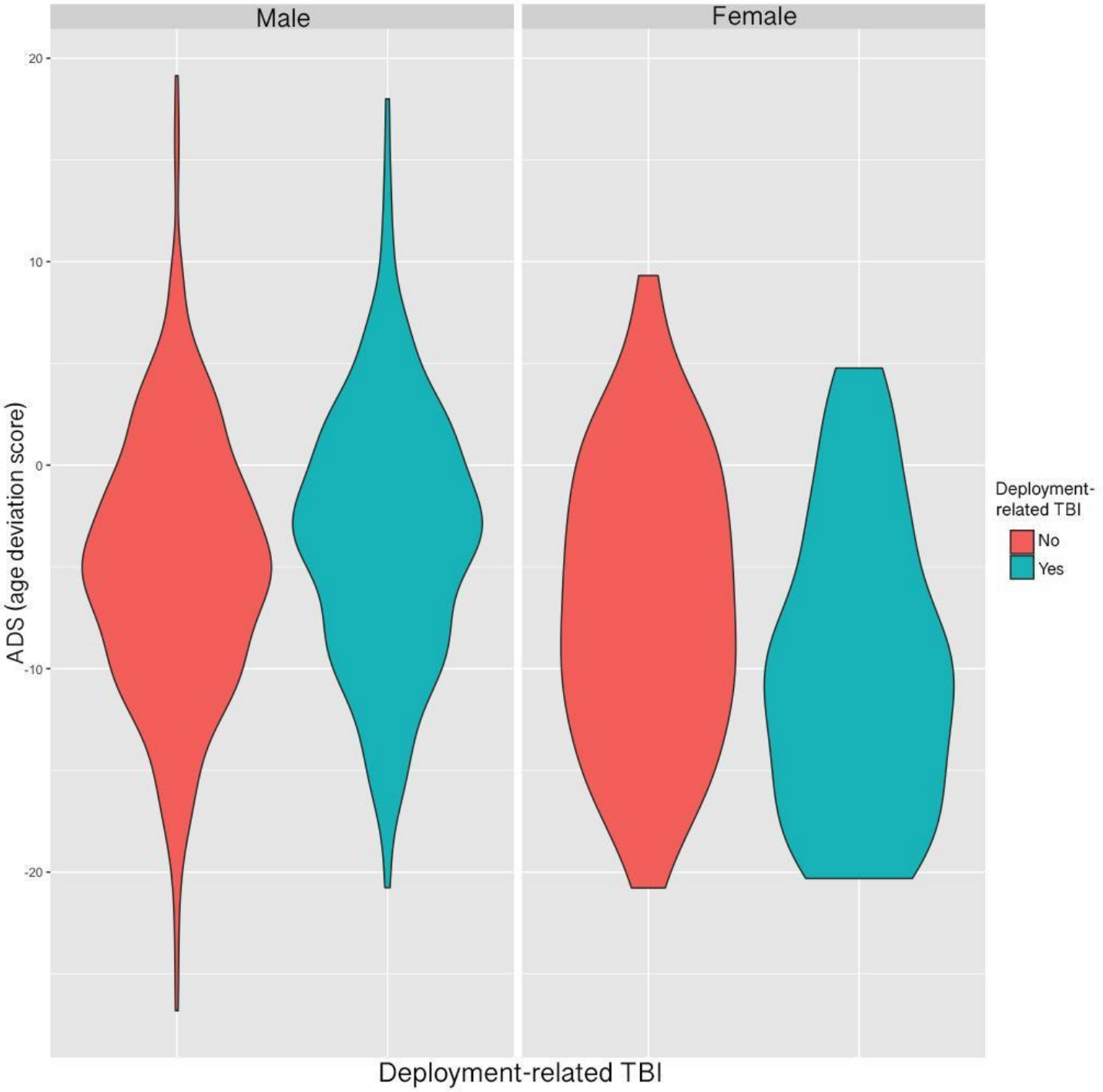

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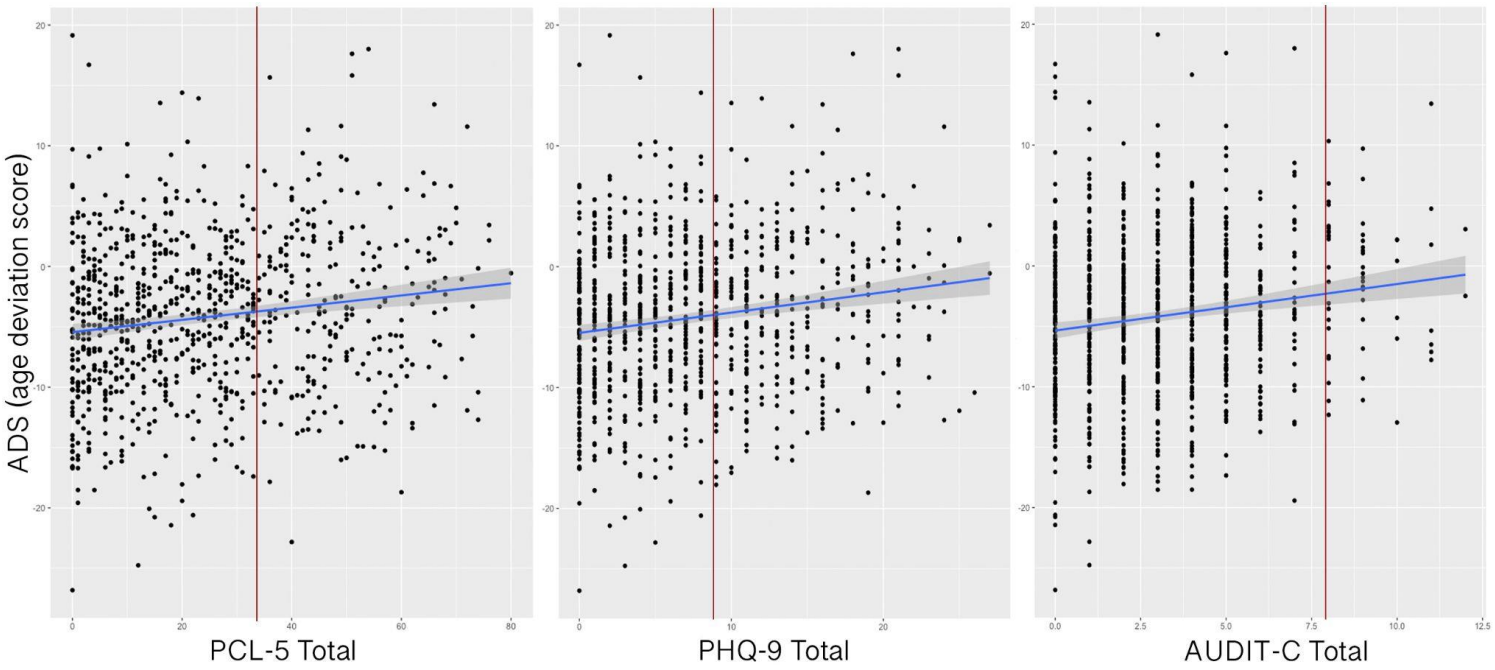

Table 2. Cognitive and Psychological Health Variables (males only). Associations between brain age and cognitive and psychological health variables among males are shown, with *T*-statistics, raw *p*-values, and FDR corrected *p*-values. *Italics* denotes $p<0.05$ prior to correction for multiple comparisons, **bold** font denotes $p<0.05$ after correction for multiple comparisons. AUDIT-C - Alcohol Use Disorders Identification Test; BVMT-R - Benton Visual Retention Test - Revised; CVLT-II - California Verbal Learning Test - Second Edition; DAST-10 - Drug Abuse Screening Test; D-KEFS - Delis Kaplan Executive Function System; PCL-5 - PTSD Checklist for DSM-5; PHQ-9 - Patient Health Questionnaire, 9-Item; WAIS-IV: Wechsler Adult Intelligence Test, Fourth Edition.

Variable	*T*-stat	Raw *p*-value	FDR corr. *p*-value
PCL-5 Total	**2.7**	**0.0067**	**0.031**
PHQ-9 Total	**3.1**	**0.0026**	**0.021**
DAST-10 Total	1.2	0.23	0.36
AUDIT-C Total	**3.0**	**0.0030**	**0.021**
BVMT-R Delayed Recall raw	*2.0*	*0.045*	*0.16*
BVMT-R Total Recall raw	1.8	0.079	0.22
CVLT-II Long Delay Free Recall raw	0.2	0.85	0.92
CVLT-II Short Delay Free Recall raw	-0.6	0.59	0.68
CVLT-II Trial Total raw	1.0	0.33	0.47
D-KEFS Category Switching Total Correct raw	1.5	0.12	0.25
D-KEFS Letter Fluency Total Correct raw	0.0	0.98	0.98
Trail Making Test (Trial B – Trial A)	1.6	0.11	0.25
WAIS-IV Digit Span raw	-0.9	0.39	0.50
WAIS-IV Processing Speed Index	-1.3	0.20	0.35

Supplementary Materials

Supplementary Table 1. T1-weighted scan protocols across the six data collection sites included in this analysis. Scanner, TR, TE, voxel size, matrix, slices, and orientation are listed for each site.

Supplementary Figure 1. Boxplots showing differences in ADS between deployment-TBI group and control group within females across 6 sites. Portland, Minneapolis, and Boston were removed from analyses for small, uneven group size.

Supplementary Table 1. T1-weighted scan protocols across the six data collection sites included in this analysis. Scanner, TR, TE, voxel size, matrix, slices, and orientation are listed for each site.

Site	Scanner	TR (ms)	TE (ms)	Voxel size (mm)	Matrix	Slices
Richmond, VA (Site 1)	Philips Ingenia	6.78	3.157	1x1x1.2	256x256	176
Houston, TX (Site 2)	Siemens TrioTim	2300	2.96	1x1x1.2	240x256	176
San Antonio, TX (Site 4)	Siemens Verio/Skyra Fit	2300	2.98	1x1x1.2	240x256	176
Portland, OR (Site 6)	Philips Achieva	6.76	3.145	1x1x1.2	256x256	170
Minneapolis, MN (Site 7)	Siemens Prisma	2400	2.24	0.8x0.8x0.8	300x320	208
Boston, MA (Site 8)	Siemens Prisma	2400	2.24	0.8x0.8x0.8	300x320	208

Supplementary Figure 1. Boxplots showing differences in ADS between deployment-TBI group and control group within females across 6 sites. Portland, Minneapolis, and Boston were removed from the female sub-analyses for small, uneven group size.