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S.I. : CONCUSSIONS II

Mild Traumatic Brain Injury and Career Stage Associate with Visible Perivascular Spaces in Special Operations Forces Soldiers

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Abstract

Mild traumatic brain injury (mTBI) and occupational blast exposure in military Service Members may lead to impaired brain waste clearance which increases neurological disease risk. Perivascular spaces (PVS) are a key part of the glymphatic system which supports brain waste clearance, preferentially during sleep. Visible PVS on clinical magnetic resonance imaging have been previously observed in patients with neurodegenerative diseases and animal neurotrauma models. The purpose of this study was to determine associations between PVS morphological characteristics, military career stage, and mTBI history in Special Operations Forces (SOF) Soldiers. Participants underwent T2-weighed neuroimaging to capture three-dimensional whole brain volumes. Segmentation was performed using a previously validated, multi-scale deep convolutional encoder-decoder neural network. Only PVS clusters within the white matter mask were quantified for analyses. Due to non-normal PVS metric distribution, non-parametric Mann–Whitney *U* tests were used to determine group differences in PVS outcomes. In total, 223 healthy SOF combat Soldiers (age = 33.1 ± 4.3yrs) were included, 217 reported career stage. Soldiers with mTBI history had greater PVS number ($z = 2.51$, $P = 0.013$) and PVS volume ($z = 2.42$, $P = 0.016$). In-career SOF combat Soldiers had greater PVS number ($z = 2.56$, $P = 0.01$) and PVS volume ($z = 2.28$, $P = 0.02$) compared to a baseline cohort. Mild TBI history is associated with increased PVS burden in SOF combat Soldiers that are clinically recovered from mTBI. This may indicate ongoing physiological changes that could lead to impaired waste clearance via the glymphatic system. Future studies should determine if PVS number and volume are meaningful neurobiological outcomes for neurodegenerative disease risk and if clinical interventions such as improving sleep can reduce PVS burden.

Keywords Blast · Concussion · Glymphatic · Military · Neuroimaging

Introduction

Traumatic brain injury (TBI) has emerged as the signature injury sustained in modern military theaters and training environments [1]. The Traumatic Brain Injury Center of Excellence reported 449,026 TBI sustained by Service

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members between 2001 and 2021, 82.3% of which were characterized as "mild" (mTBI) [2]. Special Operations Forces (SOF) face a greater risk for neurotrauma given their training demands and growing role in global conflict intervention. Estimates suggest 25–55% of SOF Service members endorse mTBI history [3–5]. These mTBI are identified by confused or disoriented states lasting more than 24 h; or loss of consciousness, if any, for more than 30 min, but less than 24 h; or memory loss lasting greater than 24 h but less than 7 days, without gross anatomical findings on standard clinical imaging. The designation "mild" is a misnomer, as mTBI are associated with persistent neurocognitive and behavioral deficits in patients [6–8]. Evidence supports a link between military mTBI history and an increased risk for atypical neurodegeneration [9]. It is imperative to determine what physiological changes following mTBI increase neurodegenerative risk so that early chronic disease detection and interventions can be developed for Service members with mTBI history.

In addition to mTBI, SOF members sustain recurrent occupational blast exposure due to a need to maintain operational readiness [10]. Although these individual exposures may not evoke mTBI symptoms, cumulative blast neurotrauma may contribute to developing long-term neurological symptoms via damage to neural tissue [11]. Progressive damage to neurons and glia which contribute to neurodegenerative changes may be caused by an inability to eliminate harmful neuroinflammatory waste caused by frequent blast exposure [12]. Recent estimates in this population indicates SOF service members are cumulatively exposed to blast overpressure during their training [10]. This cumulative exposure value could be further explored with more sophisticated algorithms related to blast frequency, severity, recency, and other ordnance systems. Notwithstanding, it highlights the essential need to detect and describe physiological changes attributed to chronic occupational blast exposure, with or without mTBI, which may lead to chronic neuropathology.

The glymphatic system is a central nervous system waste clearance pathway first described in the past 10 years [13, 14]. The glymphatic system—named for the glial cells facilitating it—shares similar functional properties to the peripheral lymphatic system. The glymphatic system uses perivascular channels to eliminate harmful cellular waste from the brain, and is believed to be most active during sleep [15, 16]. The glymphatic system is critically involved in clearing beta-amyloid and tau, which are both neurotoxic proteins that promote neurodegeneration [17–19]. Perivascular spaces (PVS) are fluid-filled spaces integral to the glymphatic system which surround perforating arterioles and venules in the brain. Normally microscopic, increased and enlarged PVS have been qualitatively observed on T2-weighted magnetic resonance imaging (MRI) as tubular white matter hyperintensities in patients with vascular dementias, Alzheimer's disease, and stroke [20, 21]. These dilated PVS have been noted as a radiologic "hallmark" of TBI, even prior to the glymphatic system's discovery [22]. Chronic glymphatic system disruption secondary to neurotrauma is suspected to have a role in linking cellular injury to adverse long-term clinical outcomes [23].

Both sleep and mTBI impact the glymphatic system. Reports indicate more than 48% of post-deployed Service members have poor sleep [24]. Increased mTBI incidence has also been associated with sleep disturbances in Service members and Veterans [25]. Enlarged PVS have been correlated with worse objective sleep metrics using polysomnography [26]. In combination, individuals with TBI show a stronger relationship between sleep and enlarged PVS compared to a non-TBI group [12]. In Veterans, mTBI was positively related to PVS volume and an interaction effect was found between mTBI and poor sleep on PVS volume [27].

Efforts have been made to quantify PVS burden using manual and automatic segmentation techniques. Most methods are time-intensive or computationally rigorous, limiting sample size or generalizability in previous studies. Advances in neural networks allow more accurate and efficient automated segmentation in larger samples. The overall study purpose was to quantify dilated PVS using a novel fully convolutional neural network and determine the association between PVS burden (number and volume) and mTBI history in SOF combat Soldiers. Our specific aims were twofold: (1) To describe the differences in PVS burden (number and volume) between SOF members with and without mTBI history and (2) describe the differences in PVS burden (number and volume) between Soldiers at the start of their SOF career (baseline) and during their SOF career (in-career). Soldiers at the baseline timepoint were individuals who exited a SOF training pipeline at a variety of age ranges and experience levels within the military. The in-career timepoint included Soldiers that matriculated and were retained by this SOF organization and sustained regular low level blast exposure due to training requirements. However, blast dosimetry was not individually tracked in this cohort. Our overall goal was to describe ongoing physiological changes that may link injury and exposure to adverse future health outcomes.

Methods

In this cross-sectional study, presently asymptomatic SOF combat Soldiers underwent multimodal neuroimaging including whole brain three-dimensional T2-weighted MRI sequences obtained on a Siemens 3T Biograph mMR or 3T MAGNETOM Prisma (TR/TE = 3200/400 ms, slice thickness = 1 mm, $FoV = 256 \times 256$ mm). All participants

completed verbal consent and study procedures were approved by the office of human research ethics at our institution. The PVS segmentation was performed using a previously validated, multi-scale deep convolutional encoder-decoder neural network [28]. Skull stripping was performed on the T2-weighted images using FSL's Brain Extraction Tool. White matter and subcortical gray matter were extracted from T2-weighted images using FMRIB's Automated Segmentation Tool (FAST) without partial volume estimation. A custom atlas with segmented cerebral white matter derived from FAST and subcortical gray matter labels from the T2-weighted JHU-MNI-ss Eve atlas were registered to each subject using Advanced Normalization Tools [28] (ANTs) to minimize misidentifying thin tubular structures in the skull, brainstem, cerebellum, and cortex as PVS. Only PVS clusters within the custom white matter mask (Figs. 1 and 2) were retained for analyses.

The morphological features of PVS burden were quantified as total PVS number and PVS volume in cubic millimeters. Self-reported mTBI history was dichotomized as "with history" and "without history." Mild TBI was operationally defined in this study as a change in brain function following a force to the head, which may be accompanied by temporary loss of consciousness, but is identified in awake individuals with measures of neurologic and cognitive dysfunction. Due to the non-normal PVS variable distribution, nonparametric Mann–Whitney *U* tests were used to determined group differences in PVS outcomes. Means (and SDs) and medians (and IQRs) were used to characterize continuous variables by group. All statistical tests were two-tailed with an a priori α level $P \leq 0.05$.

Results

In total, 223 healthy SOF combat Soldiers (mean age = 33.1, SD ± 4.3yrs) were included. Within this study sample, 123 (55.16%) self-reported mTBI history and 100 (44.84%) reported no mTBI history. Career stage data was available for 217 participants, 141 (64.9%) of which were collected at baseline. Total PVS number was significantly greater ($z = 2.51$, $P = 0.013$) for Soldiers who self-reported mTBI history (median = 40, $IQR = 49$) than for those without mTBI history (median = 33, $IQR = 35$). White matter PVS volume was also significantly greater ($z = 2.42$, $P = 0.016$) for Soldiers reporting mTBI history (median = 717 mm^3, $IQR = 1115$ mm^3) than those without mTBI history (median = 598 mm^3, $IQR = 756$ mm^3). Both PVS number and volume by mTBI history group are illustrated in Fig. 3.

In-career SOF combat Soldiers had significantly greater ($z = 2.56$, $P = 0.011$) total PVS number (median = 45.5, $IQR = 49$) than Soldiers at baseline (median = 34, $IQR = 33$) and significantly greater ($z = 2.28$, $P = 0.023$) PVS volume than Soldiers at baseline (median = 763.5 mm^3, $IQR = 1283$ mm^3 vs median = 611 mm^3, $IQR = 799$ mm^3).

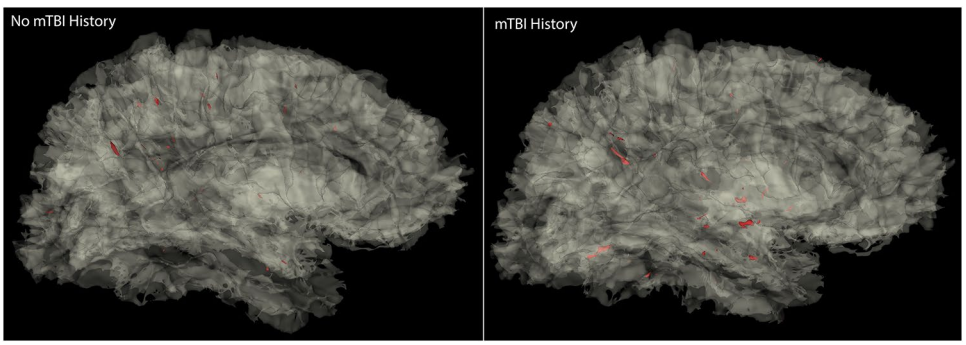

Fig. 1 Rendered 3D white matter volumes from participants in this sample representing the median PVS volume for those without mTBI history on the left and those with mTBI history on the right. PVS voxels retained for analysis within the white matter mask are colored red

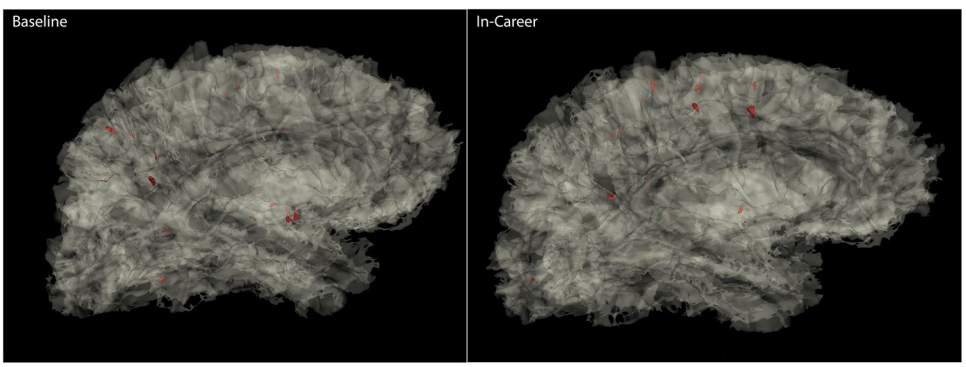

Fig. 2 Rendered 3D white matter volumes from participants in this sample representing the median PVS volume for those at Special Operations Forces career baseline on the left and those from the in-career group on the right. PVS voxels retained for analysis within the white matter mask are colored red

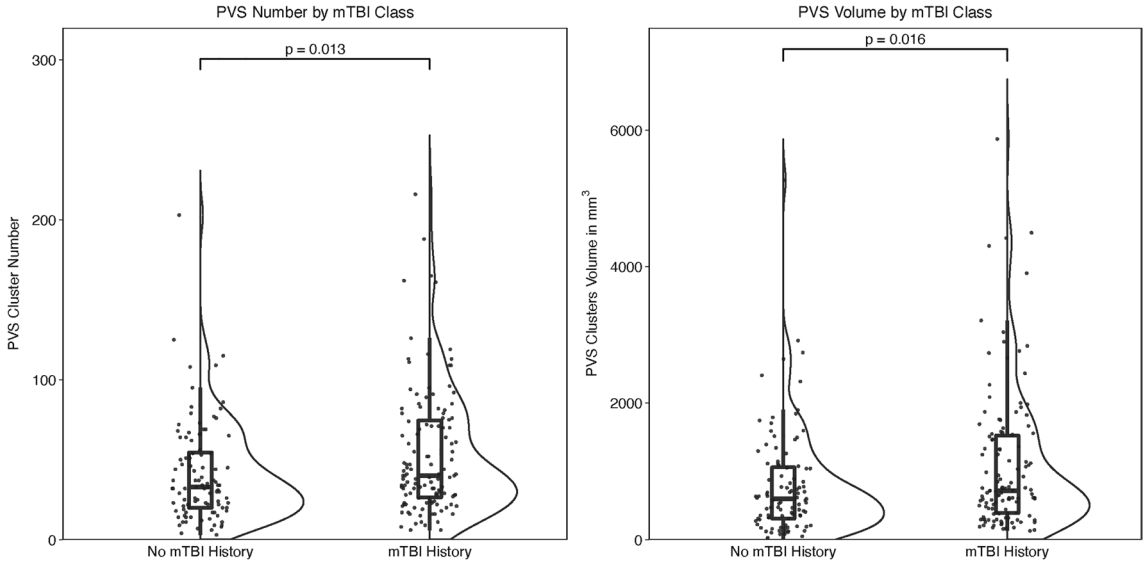

Fig. 3 Half-violin boxplots for group differences between PVS number and volume by dichotomous mTBI classification. *P* values represent Mann–Whitney *U* test significance level

Differences between PVS number and volume by career stage are illustrated in Fig. 4.

Discussion

Mild TBI history is associated with greater PVS burden (number and volume) in SOF combat Soldiers. This finding is further magnified since the participants included in our study were clinically recovered from mTBI and had fully returned to duty. Soldiers with greater occupational blast exposure, using career stage as a proxy, have greater PVS burden. This may indicate ongoing neuroinflammatory processes leading to impaired waste clearance via the glymphatic system and subsequently increased neurodegenerative disease risk.

Functional PVS are crucial for metabolic waste removal in animal studies [29]. Rodents subjected to blast and blunt mTBI show glymphatic dysfunction and aquaporin-4 channel loss [17]. Aquaporin-4 channels interface between

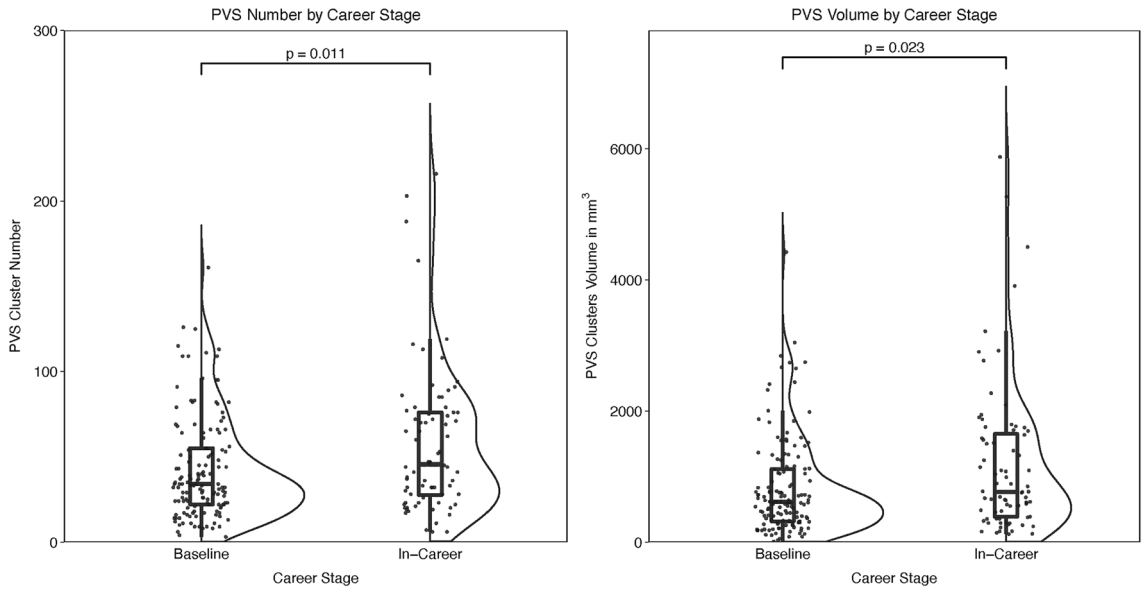

Fig. 4 Half-violin boxplots for group differences between PVS number and volume by career stage. *P* values represent Mann–Whitney *U* test significance level

astrocyte end-feet and PVS in healthy conditions, facilitating fluid exchange between the PVS and interstitial fluid [13]. Neurotrauma and neurological disorders may disrupt aquaporin-4 channel function leading to PVS dilation, impaired glymphatic flow and waste accumulation [30]. Due to the glymphatic system's role in clearing neurodegenerative mediators including beta-amyloid and tau, this waste accumulation at the PVS may spur traumatically induced neurodegenerative processes caused by neurofibrillary tangle formation and insoluble amyloid plaques [13, 17, 23].

Neuroinflammatory processes secondary to mTBI, and cell damage caused by subconcussive blast, may also cause PVS dilation. Secondary metabolic cell death occurs due to increased cellular energy demands amid lower cerebral blood flow in the sub-acute phase following mTBI [31]. Previous research in SOF combat Soldiers indicates ongoing inflammatory cell death may cause cell body factor neuron-specific enolase to remain elevated despite clinical recovery from mTBI [3]. We speculate these factors may lead to a scenario whereby the rate of glymphatic clearance is insufficient to reduce PVS burden, thereby resulting in a chronic accumulation of cell debris in the brain. However, the mechanical link between both aquaporin-4 disruption, post-traumatic neuroinflammation, and PVS enlargement is yet to be confirmed.

Limitations to this study include a reliance on self-reported mTBI history. Although this method is commonly employed in the mTBI literature, it could lead to underreporting due to ambiguous diagnostic criteria or nondisclosure. Self-reporting is commonly employed in the mTBI literature but may introduce inaccuracies or underreporting. Evidence indicates mTBI nondisclosure is prevalent in both military and civilian populations; however, we cannot reliably estimate how this might impact our sample. Several factors contribute to nondisclosure in both populations. These include fear of missing game/practice time [32], service career repercussions [33], or simply not knowing it was a concussion [32]. Nondisclosure would ultimately lead to inaccurate medical records. This limitation is not restricted to our study but, rather, to all those in our field.

Additionally, the automated novel fully convolutional neural network employed to segment PVS may be subject to mislabeling. Visual inspection and stringent white matter masking was used to mitigate any potentially false labels. Sleep is known to impact PVS morphology and mTBI status may affect sleep directly or via posttraumatic stress symptoms. We were unable to control for these relationships in the present study. Future studies should determine how sleep influences PVS burden following mTBI exposure. We also were unable to control for physiological covariates (e.g. blood pressure, intracranial pressure, weight). Relationships between PVS outcomes and neuroinflammatory biomarkers should be explored to validate a mechanistic process between cell death and glymphatic dysfunction. Longitudinal research is needed to determine if PVS number and volume are meaningful neuroimaging markers for neurodegenerative disease risk and prognosis.

Conclusion

Our sample included SOF combat Soldiers with no ongoing observable symptoms related to any previous mTBI they may have sustained. Thus, the Soldiers we studied presented for testing in a clinically 'normal' uninjured state. Noting our observations in otherwise healthy individuals may point to some longer lasting underlying physiological deficits that may persist and for which standard clinical assessments are incapable of measuring or tracking. Therefore, our finding that greater PVS burden (number and volume) was associated with mTBI history and career stage may indicate persistent post-mTBI neurophysiological changes that may affect long-term brain health.

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