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Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury

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Abstract

The complex etiology behind Gulf War Illness (GWI) has been attributed to the combined exposure to neurotoxicant chemicals, brain injuries, and some combat experiences. Chronic GWI symptoms have been shown to be associated with intensified neuroinflammatory responses in animal and human studies. To investigate the neuroinflammatory responses and potential causes in Gulf War (GW) veterans, we focused on the effects of chemical/biological weapons (CBW) exposure and mild traumatic brain injury (mTBI) during the war. We applied a novel MRI diffusion processing method, Neurite density imaging (NDI), on high-order diffusion imaging to estimate microstructural alterations of brain imaging in Gulf War veterans with and without GWI, and collected plasma proinflammatory cytokine samples as well as self-reported health symptom

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.07.006.

scores. Our study identified microstructural changes specific to GWI in the frontal and limbic regions due to CBW and mTBI, and further showed distinctive microstructural patterns such that widespread changes were associated with CBW and more focal changes on diffusion imaging were observed in GW veterans with an mTBI during the war. In addition, microstructural alterations on brain imaging correlated with upregulated blood proinflammatory cytokine markers TNFRI and TNFRII and with worse outcomes on self-reported symptom measures for fatigue and sleep functioning.

Taken together, these results suggest TNF signaling mediated inflammation affects frontal and limbic regions of the brain, which may contribute to the fatigue and sleep symptoms of the disease and suggest a strong neuroinflammatory component to GWI. These results also suggest exposures to chemical weapons and mTBI during the war are associated with different patterns of peripheral and central inflammation and highlight the brain regions vulnerable to further subtle microscale morphological changes and chronic signaling to nearby glia.

Keywords

Gulf War Illness; Veterans; Cytokines; Neuroinflammation; Diffusion imaging; MRI

1. Introduction

About a third of the nearly 700,000 U.S. troops who served in the Gulf War (GW) suffer from a complex, often debilitating symptomatic illness known as Gulf War Illness (GWI) (White et al., 2016). Symptoms of GWI typically include fatigue, chronic pain, memory and attention problems, headaches, gastrointestinal and respiratory symptoms which encompass the six symptom domains of the National Academy of Sciences recommended Kansas GWI criteria (Steele, 2000; National Academics of Sciences, Engineering, and Medicine, 2016). GWI has been associated with altered central nervous system (CNS) functioning (White et al., 2016). Chronic GWI symptoms are thought to develop as a result of a heightened innate immune response in the CNS to multiple exposures during the war including stress, neurotoxicant chemicals (organophosphate pesticides and nerve agents) and to other CNS insults, such as mild traumatic brain injury (mTBI) (Gade and Wenger, 2011; O'Callaghan et al., 2015; Rathbone et al., 2015; Yee et al., 2016; Yee et al., 2017; Janulewicz et al., 2018). mTBI as defined by the American Academy of Neurology has proven to be the most sensitive measure of mTBI in prior GWI research (Vynorius et al., 2016; Yee et al., 2016; Yee et al., 2017; Janulewicz et al., 2018). As such, the persisting symptoms of GWI have been hypothesized to coincide with a heightened, chronic neuroinflammatory reaction observed in animal models while increased blood levels of proinflammatory cytokines in veterans with GWI has also been reported (Whistler et al., 2009; O'Donovan et al., 2015, O'Callaghan et al., 2015; Khaiboullina et al., 2014; Parkitny et al., 2015; Locker et al., 2017; Koo et al., 2018; Miller et al., 2018; Janulewicz et al., 2019). However, we are not aware of any publications to date examining microstructural integrity and neuroinflammatory responses by utilizing brain imaging techniques to focus on mTBI and organophosphate (OP) exposure in GWI veterans.

Exposure to neurotoxicants including OP pesticides and sarin nerve agents has been a unique risk factor associated with GWI (Golomb, 2008; White et al., 2016; Sullivan et al., 2017). In animal models, exposure to OP nerve agents and pesticides, such as sarin and its surrogate diisopropyl fluorophosphate (DFP) and chlorpyrifos, was shown to produce neuroinflammation as indicated by increased proinflammatory cytokine signaling in the brain (Spradling et al., 2011; O'Callaghan et al., 2015; Locker et al., 2017). Neuroinflammatory cytokines were further associated with microstructural changes in the brain in the OP-exposed animal model of GWI indicating potential damage associated signaling and activation of proinflammatory cytokine release from nearby glia (Banks and Lein 2012; Koo et al., 2018).

These microstructural changes need not reflect neuronal damage or apoptosis but could rather reflect more subtle microscale morphological changes including dendritic or glial cell arborization (Spradling et al., 2011; Koo et al., 2018). In humans, brain morphometric analysis based on T1 weighted magnetic resonance imaging (MRI) scans of GW veterans exposed to the chemical weapon sarin showed overall reductions in grey matter (GM) and selective reductions in hippocampal subfield volumes when compared with unexposed veterans (Chao et al., 2011, 2015). In the white matter (WM), an overall reduction in tissue volume was observed in a dose–response manner in GW veterans with air plume-modeled exposure to sarin (Heaton et al., 2007). These WM volumetric changes in sarin exposed veterans have also been validated in other cohorts and correlated with cognitive outcomes (Proctor et al., 2006; Chao et al., 2010). More recently, two investigations using diffusion tensor imaging (DTI) have reported altered brain connectivity which correlated with fatigue, pain, or hyperalgesia in GW veterans with sarin exposure and in those with GWI (Rayhan et al., 2013; Chao et al., 2015). In both studies, enhanced axial diffusivity in the major WM tract pathways was suggested as a potential biomarker for GWI and was associated with more severe health symptom reporting (Rayhan et al., 2013; Chao et al., 2015). These findings indicate a structure–function relationship between WM changes and chronic health symptoms in GW veterans that may be related to chronic microglial activation and neuroinflammatory cytokine signaling from damaged neural cells including more subtle neurite microstructural alterations signaling to nearby glia (O'Callaghan et al., 2015; Banks and Lein, 2012; Rathbone et al., 2015).

Mild traumatic brain injury (mTBI) is another factor that can produce a secondary neuroinflammatory response post-injury (Kumar and Loane; 2012, Rathbone et al., 2015). mTBI is the most common type of traumatic brain injury affecting military personnel. More than 15 percent of returning members experienced mTBI (Hoge et al., 2008) and it has recently been shown to be highly prevalent (~30%) in the large, longitudinally-followed Ft. Devens cohort of GW veterans and in the Boston Gulf War Illness Consortium (GWIC) cohort of GW veterans (Hoge et al., 2008; Yee et al., 2016; Janulewicz et al., 2018). Increasing evidence suggests that a single mTBI may produce long-term progressive damage in GM and WM, and accelerate age-related neurodegeneration and neuroinflammatory signaling (Bramlett and Dietrich, 2002; Smith et al., 2013; Rathbone et al., 2015; Chao, 2018). In addition, it has recently been shown that GW veterans with a mTBI history alone or in addition to sarin chemical weapons (CBW) exposure during the war are more likely to report persistent and debilitating chronic health symptoms and medical conditions

suggesting that multiple mTBIs or a single mTBI and chemical weapons exposure act as multiple-hits to the neuroimmune system that primes stronger and longer neuroinflammatory signaling in those exposed (Yee et al., 2017; Janulewicz et al., 2018; O'Callaghan and Miller 2019). However, brain imaging outcomes in GW veterans with mTBI and with chemical weapons exposures during the war and their effect on microscale morphological changes including dendritic or glial cell arborization and neuroinflammatory signaling have yet to be reported.

We have previously demonstrated that high-order diffusion MRI showed a sensitivity to discriminate different stages of neuroinflammatory signaling in our established, OP exposed GWI animal model utilizing combined exposure to exogenous corticosterone at levels mimicking high physiological stress and the sarin surrogate, DFP (Koo et al., 2018). When combined with findings from other similar animal model studies, results suggest a strong brain-immune component to GWI that could be measured through brain imaging and peripheral blood immune markers and validated in GW veteran cohorts (O'Callaghan et al., 2015, Spradling et al., 2011).

Neurite density imaging (NDI, Zhang et al., 2012) and Q-space imaging (Yeh et al., 2010) are two novel diffusion processing methods of the high-order diffusion MRI measures that have been shown to successfully detect local microscale diffusivity of axon and dendrite processes in animals and human studies of neurological disorders (Colgan et al., 2016, Zhang et al., 2012, Koo et al., 2018, McCunn et al., 2019). NDI compartmentalizes the brain environment into three components to sample microstructural diffusivity, and restricted diffusion imaging measure (RDI) in Q-space imaging method provides diffusion displacement in the three-dimensional space that could provide similar diffusion information of NDI by analyzing different boundaries in the three-dimensional space (Zhang et al., 2012; Yeh et al., 2010, 2017). Both NDI and RDI can provide detailed description of microscale diffusivity of brain tissues and nearby free water space without applying predefined linear diffusion models as seen in conventional DTI approaches (Tuch, 2004; Zhang et al., 2012). Decomposing slow diffusion components with links to subneuronal, glial or extracellular compartments may give detailed insights on pathophysiologic profile of disease.

In this study, we investigate whether an NDI processing model of high-order diffusion MRI can successfully identify and validate the different levels of microstructural and macrostructural brain alterations previously seen in animal models of GWI by utilizing RDI (Koo et al., 2018) and assessing how these patterns overlap in veterans with GWI from the Boston Gulf War Illness Consortium. We also assessed the relationship between brain imaging measures, blood neuroinflammatory markers, and self-reported health symptoms in veterans with GWI and GW control veterans. Lastly, we compared the separate and combined effects of mTBI and chemical weapons exposure on high-order microstructural diffusion MRI, blood neuroinflammatory markers, and health symptom outcomes.

2. Materials and methods

2.1. Participants

The study population included 91 GW veterans from the Boston University Gulf War Illness Consortium (GWIC). The GWIC is a multi-site study that includes a series of preclinical and clinical studies designed to understand the pathobiological mechanisms responsible for the chronic symptoms of GWI and to identify diagnostic markers and targeted treatments for the disorder. GWIC inclusion criteria required deployment to the Persian Gulf between August 1990 and July 1991. GWIC exclusion criteria included diagnoses of chronic medical illnesses that could otherwise account for the symptoms experienced by GW veterans. These diagnoses included autoimmune, central nervous system, or major psychiatric disorders that could affect brain and immune functions (e.g., epilepsy, stroke, severe head injury, brain tumor, multiple sclerosis, Parkinson's disease, Alzheimer's disease, schizophrenia, bipolar disorder, and autoimmune disorders). Each of the study participants completed an assessment protocol including health surveys, a neuropsychological test battery, brain imaging, and collection of blood and saliva samples (Janulewicz et al., 2018). All participants provided written informed consent to participate in the study. This study was reviewed and approved by the Boston University institutional review board.

2.1.1. Gulf war illness criteria—GWI case status was defined from the Kansas GWI case definition (Steele, 2000). The Kansas GWI case definition requires GWI cases to endorse multiple or moderate-to-severe chronic symptoms in at least three of six statistically-defined symptom domains: fatigue/sleep problems, somatic pain, neurological cognitive/mood symptoms, gastrointestinal symptoms, respiratory symptoms and skin abnormalities (Steele, 2000). GWIC participants not meeting Kansas GWI or exclusionary criteria were considered controls. Veterans were excluded from being considered GWI cases, for purposes of the research study, if they reported being diagnosed by a physician with medical or psychiatric conditions that would account for their symptoms or interfere with their ability to report their symptoms.

2.1.2. Self-Reported mild traumatic brain injury (mTBI)—To determine mTBI status, participants were given a concussion definition that follows the current guidelines from the American Academy of Neurology and was used in our prior GW veteran mTBI publications (Vynorius et al., 2016; Robbins et al., 2014; Seichepine et al., 2013; Janulewicz et al., 2018; Yee et al., 2016; Yee et al., 2017). Participants were provided with the mTBI definition and examples of common symptoms associated with mTBI and were then asked to report if they had experienced mTBI during their deployment, they were also asked to self-report how many mTBIs they had experienced during the war.

2.1.3. Chemical/Biological weapon (CBW) exposure—GWIC subjects were administered the Kansas Gulf War Experiences and Exposure Questionnaire, and the Structured Neurotoxicant Assessment Checklist (SNAC) to assess for deployment-related exposures (Proctor et al., 1998; Steele 2000; Proctor et al., 2006). Self-reported exposures to chemical or biological weapons (CBWs) were obtained from the SNAC by asking the

veterans whether or not they were exposed to CBWs during military service (Proctor et al., 1998).

2.1.4. Demographics and health symptom surveys—GWIC subjects were also administered a general demographic information and medical conditions questionnaire and the Kansas Gulf War and Health Questionnaire (Proctor et al., 1998; Steele 2000). Additional validated health symptom surveys were completed by study participants and included the Multidimensional Fatigue Inventory (MFI-20), McGill Pain Inventory and the Pittsburgh Sleep Quality Index (PSQI) where higher scores indicated more symptoms (Buysse et al., 1989; Smets et al., 1995; Melzack, 1975).

2.1.5. Cytokines—EDTA plasma was separated and stored at − 80 °C until assayed. Cytokines were measured with an 18-multiplex chemiluminescent assay using Quansys Q-view Imager LS 1.3 and reagents in methods previously reported (Fletcher et al., 2009). Each 18-multiplex plate was imaged at 500 sec, 270 sec, 180 sec, 120 sec. Following the manufacture's protocol, the 270-sec images were used for further analysis. All plates were normalized by using an internal plasma control (pooled plasma from 50 men and 50 women). This internal control (IC) was run on each plate, average pg/ml was calculated for IC across plates and each plate normalized to the percent change from IC average. This normalization removes variability between plates. In instances when the cytokine expression was below the level of detection (BLD), the difference between the lower limit of detection and 0 was used. To determine if circulating proinflammatory cytokines levels were different between GWI cases and controls, plasma samples were examined by symptom group. In this study, chemiluminescent imaging concentrations of three cytokines in plasma samples were examined and compared to the brain imaging measures. Cytokines of interest were Interleukin 1 alpha (IL1α), Tumor necrosis factor receptor type I (TNFRI) and Tumor necrosis factor receptor type II (TNFRII) based on previously demonstrated relationships between GWI and blood cytokine measures (Jaundoo et al., 2018; O'Callaghan et al., 2015; Khaiboullina et al., 2014; Broderick et al., 2011).

2.2. Image acquisition

All MRI scans were performed on an Achieva 3 T whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) in the center of biomedical imaging, Boston university school of medicine.

2.2.1. T1 MPRAGE Acquisition: The Alzheimer's disease neuroimaging initiative (ADNI)—developed an MPRAGE sequence that was used for this study (TR = 6.8 msec, TE = 3.1 msec, flip angle = 9°, slice thickness = 1.2 mm, 170 slices, FOV = 250 mm, matrix = 256 × 256). We used the MPRAGE scan to generate the anatomical regions of interest (ROI) for assessing morphometric differences between the groups and also to provide anatomical co-registration with the DTI and fMRI data sets.

2.2.2. Diffusion MRI: The diffusion MRI data were obtained using a single-shot EPI sequence—with multi-shell diffusion encoding (b-value used = 1000, 2000, and 3000 s/mm2). We used 124 gradient directions utilizing parallel imaging on a 16-channel

parallel head coil (70 slices, TR = 13214 msec, TE = 55 msec, with a matrix size of 128 × 128 yielding a resolution of 2.0 × 2.0 × 2.0 mm^3, no slice gap). In addition to distortion corrections built into the scanner, we also collected 6 B0 field maps for further distortion correction.

2.3. Image processing and anatomical defining

2.3.1. Defining GM anatomy—Defining anatomical structures in the cortex was the first step in analyzing brain images. MPRAGE structural scans were analyzed using FreeSurfer (Fischl, 2012) to obtain measures of volume, cortical thickness and surface geometry for each anatomical ROIs implemented in the brain atlas (Desikan et al., 2006). Seventy-eight ROIs defined in the average template space were co-registered to each subject's cortical surface by applying nonlinear coregistration parameters. The results were visually inspected for artifacts or incomplete segmentation. A total of seventy-eight cortical and subcortical ROIs were chosen for the analysis.

2.3.2. Defining WM anatomy—Diffusion MRI was registered to the structural MRI following the motion and eddy current distortion correction (Jenkinson et al., 2012). TRACULA (TRActs Constrained by UnderLying Anatomy) software was used to perform tract-based analysis on the preprocessed diffusion MRI data (Yendiki et al., 2011). Eighteen major white matter tracts were reconstructed for each subject.

2.4. High-order diffusion processing

To reconstruct microstructural information from high-order diffusion MRI, Neurite Density Imaging (NDI) processing was performed on merged high-order diffusion MRI images containing 3 different b-value encodings (Zhang et al., 2012). NDI applies a two-level approach by separating the volume fraction of Gaussian isotropic diffusion, representing the cerebrospinal fluid (CSF) water component. Then, the remaining diffusion signal is sub-compartmentalized into components from intra and extra-neurite water (Zhang et al., 2012). This modeling procedure provides a neurite density (ND) index, a fraction of tissue composed of axons or dendrites, and the fraction of tissue other than neurites. Orientation dispersion (OD) index provides the spatial configuration of the neurite structures based on the composite pattern of intra and extracellular diffusivity. Both ND and OD measures in each voxel were merged into 18 WM major tracts to extract tract-wise measures. For the GM and subcortical GM diffusivity assessment, diffusion modeling parameters were determined by iterative parameter selection methods based on the maximum likelihood estimation of modeling fitting error. These three different measures from this step were then merged into the 78 GM ROIs to extract ROI-wise NDI measures.

2.5. Statistical analysis

Group differences on ROI levels between GW veteran controls (GW Cont) and veterans with GWI (GWI Case) were assessed by generalized linear regression models controlling potential confounding variables such as age and gender (Gur et al., 1991). Significant p-values ($p < 0.05$) were first calculated through nonparametric permutation tests with 10,000 permutations (Winkler et al., 2014), then we applied the Benjamini & Hochberg procedure to control the false discovery rate (FDR) (Benjamini and Hochberg, 1995; Groppe et al.,

2014). Significant p-values after permutations (p) or FDR adjustment (FDR_adj_p) in the whole GM and WM group comparisons were reported along with *t*-values.

Partial correlations controlling for age and gender were applied on:

1. Multidimensional Fatigue Inventory scale (MFI) and GM NDI data;
2. Pittsburgh Sleep Quality Index (PSQI) sleep score and GM NDI data;
3. plasma blood cytokine data and GM NDI data. Both whole group and subgroup level analyses were assessed in this study. Significant p-values after permutations (p) or FDR adjustment (FDR_adj_p) in the whole group and subgroup levels were reported along with the Pearson correlation coefficients (rho). For subgroups analyses with small sample sizes, we included 95% confidence intervals (95% CI)

3. Results

3.1. Demographic results

The first 91 GWIC veterans with brain imaging completed were the participants in this study. 75 GW veterans met Kansas criteria for GWI (GWI Cases) and 16 GW veterans did not meet Kansas GWI criteria and were considered GW veteran controls (GW Controls). Veterans with GWI were further divided into subgroups based on self-reported exposures to chemical weapons (CBW) or mTBI during their deployment. Those exposed to mTBI during deployment (GWI + mTBI; n = 23), CBW agents (GWI + CBW; n = 33) or both exposures (GWI + mTBI + CBW; n = 12) (Table 1).

3.2. GWI decreases NDI measures in both WM and GM regions

Whole group analysis in both WM and GM imaging measures indicated significant differences between GWI cases and controls, with p-values < 0.05 after FDR correction (Fig. 1, Sup.1, Sup.2).

Compared to controls, significantly decreased patterns in GWI cases were seen in ND for all major WM tracts. Both ND and OD showed decreased patterns for most GM ROIs. The highest significant group differences between GWI cases and controls were seen in the left cingulum angular bundle (cab, t = −2.963, FDR_adj_p = 0.027), the bilateral uncinate fasciculus (unc, t = −2.749, FDR_adj_p = 0.026 (left), t = −2.941, FDR_adj_p = 0.026 (right)), the bilateral rostral anterior cingulate (t = −3.272, FDR_adj_p = 0.026 (left), t = −2.882, FDR_adj_p = 0.026 (right)), and the bilateral fusiform gyrus (t = −3.006, FDR_adj_p = 0.026 (left), t = −2.909, FDR_adj_p = 0.026 (right)) (Fig. 1, Sup.1, Sup.2).

3.3. GWI subgroups have distinct patterns of behavioral symptoms and brain changes

Specific risk factors were selected to define subgroups for correlation analysis to self-reported health symptom measures. GM ND and self-reported symptom scores within mTBI, CBW and mTBI + CBW subgroups showed an overall negative relationship, but highlighted specific regions in each subgroup (Fig. 2, Sup. 3, Sup. 4). There were more localized patterns in GWI + mTBI ND and OD measures, with the most significant results

seen in the left pars orbitalis for the MFI score (rho = −0.706, FDR_adj_p = 0.027, 95% CI = [−0.859, −0.389]) and the left lingual gyrus for the PSQI score (rho = −0.709, FDR_adj_p = 0.036, 95% CI = [−0.860, 0.374]) (Fig. 2, Sup. 3, Sup. 4). Conversely, the GWI + CBW subgroup has more widespread and bilateral patterns for both ND and OD, some of the most significant results seen in the bilateral rostral anterior cingulate for the MFI score (rho = −0.655, FDR_adj_p = 0.002, 95% CI = [−0.803,−0.373] (left), rho = −0.605, FDR_adj_p = 0.002, 95% CI = [−0.771,−0.297] (right)) and the bilateral caudal anterior cingulate for the PSQI score (rho = −0.520, FDR_adj_p = 0.038, 95% CI = [−0.688, −0.129] (left), rho = −0.493, FDR_adj_p = 0.038, 95% CI = [−0.779, −0.316] (right)) (Fig. 2, Sup. 3, Sup. 4). The GWI + mTBI + CBW group showed enhanced patterns in restricted regions found in the single risk factor subgroup analysis, with the most significant results seen in the bilateral caudal middle frontal gyrus (rho = −0.804, FDR_adj_p = 0.036, 95% CI = [−0.949, 0.476] (left), rho = −0.808, FDR_adj_p = 0.036, 95% CI = [−0.951, 0.491] (right)) for the MFI score and the right parahippocampal gyrus for the PSQI score (rho = −0.698, p = 0.036, 95% CI = [−0.919, −0.194]) (Fig. 2, Sup. 3, Sup. 4).

3.4. Peripheral immune markers are associated with decreased NDI measures

Plasma cytokine markers showed negative relationships with NDI measures within the subgroups (Fig. 3, Sup. 5, Sup. 6, Sup. 7). Specifically, in the GWI + mTBI group, TNFRI and TNFRII showed significant negative correlations with the left entorhinal cortex (TNF RI: rho = −0.439, p = 0.041, 95% CI = [−0.707, −0.006]; TNF RII: rho = −0.523, p = 0.015, 95% CI = [−0.758, −0.115]) and the left parahippocampal gyrus (TNF RII: rho = −0.461, p = 0.036, 95% CI = [−0.735, −0.063]) regions (Sup. 5, Sup. 7). Additionally, partial correlation analysis of IL1A revealed the most significant relationship with the left middle temporal gyrus (rho = −0.567, p = 0.008, 95% CI = [−0.804, −0.229]) (Sup. 5, Sup. 7). In the GWI + CBW group, TNFRII had significant negative correlations with many bilateral cortices including the entorhinal, cingulate, parahippocampal, thalamus, occipital and temporal regions. The bilateral entorhinal cortices had the most significant negative correlation to TNFRII (rho = −0.525, p = 0.002, 95% CI = [−0.721, −0.192] (left), rho = −0.418, p = 0.017, 95% CI = [−0.669, −0.093] (right)) (Fig. 3, Sup. 5, Sup. 6).

4. Discussion

This study showed that the NDI model of high-order diffusion MRI processing detected detailed microstructural alterations in WM tracts and GM ROIs in veterans with GWI, which validated results from our previous work utilizing the GWI rat model where neuroinflammation, as measured by increased brain cytokine signaling, was correlated with high-order diffusion MRI in toxicant-exposed animals (Koo et al., 2018). Our major findings are 1) Veterans with GWI showed widespread microstructural changes compared to control veterans in both ND and OD measures, with the most pronounced differences in the frontal white matter tracts and the limbic/paralimbic cortical regions, 2) Veterans with more pronounced brain changes reported higher rates of exposure to mTBI and CBW during their deployment, 3) Veterans with CBW exposure showed widespread microstructural brain changes while those with mTBI showed more focal microstructural changes on high-order diffusion MRI. 4) Behavioral symptoms were associated with distinct brain changes across

the GWI exposure subgroups, and 5) Peripheral immune cytokine markers correlated with increased fatigue and sleep symptoms and with brain NDI measures in veterans with GWI indicating structure–function relationships between brain imaging, inflammatory markers, and behavioral outcomes.

The tissue water diffusion information captured in diffusion MRI can be potentially sensitive to many factors including axons, dendrites as well as myelinated fibers, changes in the neuroglial cells may also be a potential factor for differential patterns in water diffusivity (Gulani et al., 2001; Naughton et al., 2018; Belgrad et al., 2019). Water diffusivity may differ from either loss of existing neurons or reproduced neurons (neurogenesis) in the tissue medium. Also, changes in morphology in neuroglial cells take place during different stages of activation thereby resulting in differential patterns of water diffusivity in the brain (Raivich et al., 1999). Considering all these components, variations in the tissue environment might be expressed in a mixture of diverse diffusion strengths. A significant loss in cell populations can impact fast (i.e., macroscopic) water diffusion components since there will be less barriers for restricting water diffusion in the cell medium (Johnson et al., 2014). On the other hand, changes in sub-neuronal components, such as synaptogenesis or glial activation, can increase complexity in the medium and thereby change distinct diffusion components compared to the neuronal loss (Zhuo et al., 2012). While DTI measures could provide overall information of microstructural tissue changes in the brain, common markers of DTI, mean diffusivity (MD) and fractional anisotropy (FA), take in account of changes in all tissue components, hence novel approaches such as NDI and RDI could provide more specific information on the aforementioned changes in different tissue components as well as fiber orientation estimation (Tuch, 2004; Zhang et al., 2012)

OP nerve agents induce neuroinflammatory responses in cortical structures including limbic and paralimbic structures (Spradling et al., 2011; Rao et al., 2017; Naughton et al., 2018). Such neuroinflammatory responses might result from neurological damage as a result of neurotoxicant exposure and damage signaling to innate immune cells (Milligan and Watkins, 2009). However, the level of damage might also show mild long-lasting changes in sub-neuronal components and morphometry of neurite cells including axons and dendrites rather than the remarkable loss of neurons (Spradling et al., 2011; O'Callaghan et al., 2015). The lower range of diffusion encodings used in diffusion MRI (typically, around b = 1000 s/mm^2) is the most common protocol in clinical imaging. Under this protocol, diffusion MRI has been a powerful tool for assessing WM major pathways, edema, or brain tumors. However, it does not have enough sensitivity to assess the mild progressive damage in the sub-neuronal components since the sub-neuronal component alterations including axonal microtubule density and stability changes, myelin depletion and oligodendrocyte function and arborization of dendrites or glial process morphometry changes might induce changes in variant forms of microscopic water diffusivities (Rao et al., 2017; Naughton et al., 2018; Belgrad et al., 2019). In our previous study on GWI animal model brain imaging, we confirmed neurotoxicant-induced neuroinflammatory response accompanies microscale changes in the neuronal cell environment that significantly correlated with proinflammatory cytokine signaling (Koo et al., 2018). These results also highlight the ability to detect inflammatory-induced changes in microstructural diffusion imaging. The results from our

previous work were the rationale for studying separate diffusion components on brain imaging in GW veterans with various exposures and peripheral cytokine markers.

Based on the high-order diffusion MRI, we have confirmed that the NDI successfully and significantly differentiated between veterans with and without GWI. While NDI measures revealed overall and widespread pattern differences between groups, the clearest distinctive pattern was confirmed in the limbic/paralimbic structures along with the anterior WM connections. However, little significant differences were observed in DTI measures in major WM tracts (Sup. 11). In addition to WM, GM diffusion mapping provided a clear explanation of the relationship between microstructural damage and illness symptoms. Considering the cytoarchitectural profiles of the cortical structures, GM measures from high-order diffusion MRI may reflect distinct patterns of microstructural damage across regions. As previously discussed (Glasser et al., 2014), neuronal density in brain regions co-varies with myelinated axons. While NDI could be sensitive to myelinated axons (Fukutomi et al., 2018; Grussu et al., 2017), lowered ND in both medial prefrontal regions and anterior WM tracts may reflect damage in myelinated axons. However, other regions had more dominant changes in GM than in the WM. The cingulate cortex and parahippocampal area have relatively thick cortical layers and unmyelinated fibers. These regions may account for different neurological sources for NDI mapping. Similar to what we have confirmed from the animal model of GWI using RDI measure (Koo et al., 2018), we have found a strong link between NDI measures and RDI measure on the GW human data used in this study, suggesting NDI profiles may also account for neuroinflammatory responses in the brain (Sup. 11). Indeed, some of our NDI mappings, such as the precuneus and the anterior cingulate cortex, have overlapped patterns to those of a recent GWI study using the translocator protein (TSPO) based positron emission tomography imaging (Alshelh et al., 2020). This may indicate that NDI contrasts can be affected by activated glial cell populations in local brain regions.

Multiple risk factors have been investigated in search of the underlying causes of GWI symptoms, suggesting a neuroinflammatory etiology due to individual or multiple neurotoxicant exposures during deployment (White et al., 2016; Abou-donia et al., 2017; Sullivan et al., 2017). Recent studies have identified mTBI to play a significant role in increased rates of health-related symptoms (Yee et al., 2016; Yee et al., 2017, Chao, 2018; Janulewicz et al., 2018) in GW veterans whereas OP chemical warfare agents were critical risk factors to GWI symptoms specifically (Chao et al., 2010, 2011, 2015). Besides, high-order diffusion MRI has previously been shown to detect microstructural changes in a rat model of mTBI (Zhuo et al., 2012). As a result, we focused on GWI cases with either one or both of those risk factors as separate subgroups for further analysis and to recapitulate existing results. In this study, mTBI groups showed more focal diffusion changes while the CBW exposed group showed more widespread diffusion changes in the WM tracts and the GW ROIs. Similar to what we confirmed with GWI animal models, this may indicate that microscale changes in the neuronal cell environment can be a potential biomarker for explaining illness symptoms in GWI and groups with specific brain insults (physical and chemical) during the war (Koo et al., 2018). However, further testing in a large scale sample is needed to draw integrative and generalizable conclusions.

4.1. Behavioral symptoms and associated brain changes

Due to the complex, multi-symptomatic etiology of GWI, various clinical and self-reported symptom measures were used in our correlation analysis to investigate the relationship between imaging results and symptom severity. Overall, subjects with more depleted ND and OD reported worse sleep quality on PSQI and higher fatigue levels on the MFI indicating objective markers for subjective symptom complaints. We observed the most significant correlation between imaging data and MFI scores indicated a strong CNS component to fatigue in GWI. Fatigue symptoms showed strong associations with decreased parahippocampal measures, which is consistent with previous studies on GM volumes in other disorders including chronic fatigue syndrome (Puri et al., 2012; Tang et al., 2015; Kimura et al., 2019). Limbic and nearby related paralimbic areas had the most altered GM integrity and also displayed the most significant negative relationships among all regions in addition to the particular regions responsible for each symptom.

4.2. TNF mediated inflammation

Proinflammatory cytokine levels in the blood could be used as markers to indirectly analyze CNS innate immune responses after exposures or experiences to noxious external stimuli, which in GWI studies were often chemical warfare agents and exposures to similar classes of chemicals (Michalovicz et al., 2019). Exposure to neurotoxicants such as sarin, PB, pesticides, and other chemical warfare agents has been identified to pose negative health effects in GW veterans in cohort studies (Chao et al., 2010, 2011,2015; Sullivan et al., 2003; Sullivan et al., 2017; Zundel et al., 2019) and controlled animal studies (Abdullah et al., 2011). Indeed, the GWI + CBW group displayed significantly upregulated TNF RI and TNF RII along with decreased ND in frontal and subcortical limbic regions, similar regions highlighted with symptom-specific domains. The main ligand for both TNF RI and TNF RII, TNFα is a potent inflammatory cytokine released by macrophages triggering numerous events including apoptosis, edema, and leukocyte adhesion (Zelová and Hošek, 2013). Receptor shedding has been proposed as a mechanism to counteract high levels of TNFα to balance inflammatory responses (Xanthoulea et al., 2004; Hawari et al., 2004). Previous studies have shown TNFα to be a significant biomarker for GWI (Broderick et al., 2011; Khaiboullina et al., 2014; O'Callaghan et al., 2015; Jaundoo et al., 2018). However, unlike what we confirmed from TNF RI and RII, we did not see significant patterns in TNFα in this study. The discrepancy between these measures should be determined in further studies to clarify the role of the TNF pathway in mediating inflammation, which may contribute to the fatigue and sleep symptoms of the disease.

5. Conclusion

Our study provides neuroimaging evidence underlying GWI etiologies and reveals GWI-specific microstructural changes in the frontal and subcortical paralimbic regions due to mTBI and chemical weapons exposures. We showed for the first time in GW veterans that mTBI was associated with discrete focal microstructural changes on MRI and that chemical weapons exposures resulted in more diffuse and widespread microstructural changes on brain imaging. In addition, these microstructural brain changes correlated with peripheral neuroinflammatory markers in the blood of veterans with GWI. When these results are

combined with our prior studies showing correlations with brain cytokines and microstructural changes in the GWI animal model, this provides compelling evidence for neuroinflammation in the pathobiology of GWI. This is especially the case given that the NDI microstructural brain changes also negatively correlated with the self-reported markers of fatigue and sleep on the MFI and PSQI which suggests functional consequences from these structural changes and also validates their use as objective measures and validating NDI imaging as a potential marker of treatment trial efficacy pre- and post-treatment for GWI symptoms. Correspondingly, current GWI literature on microstructural alterations due to neuroinflammation in the limbic areas have indicated changes in memory and emotion-related functions as evidenced by psychological and health outcome correlational studies (Toomey et al., 2009; Chao et al., 2010; Abdullah et al., 2011; Chao et al. 2011; Sullivan et al., 2003; Janulewicz et al., 2018; Sullivan et al., 2017; Jeffrey et al., 2019). However, there are several limitations to human studies, which can be overcome with concurrent controlled animal experiments as we have done in our ongoing GWIC studies (O'Callaghan et al., 2015; Koo et al., 2018). Further studies are needed to elucidate which neuronal and glial changes are contributing to diffusion imaging results seen here and how microstructural alterations may lead to higher risks of accelerated aging and earlier risks for neurodegenerative and cerebrovascular diseases in GW veterans so that intervention strategies can be implemented (Barnes et al., 2018; Smith et al., 2013; Zundel et al., 2019).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

Abdullah L, Crynen G, Reed J, Bishop A, Phillips J, Ferguson S, Mouzon B, Mullan M, Mathura V, Mullan M, Ait-Ghezala G, Crawford F, 2011 Proteomic CNS profile of delayed cognitive impairment in mice exposed to gulf war illness. NeuroMol. Med 13, 275–288.

Abou-Donia MB, Conboy LA, Kokkotou E, Jacobson E, Elmasry EM, Elkafrawy P, Neely M, Bass CR, Sullivan K, 2017 Screening for novel central nervous system biomarkers in veterans with Gulf War Illness. Neurotoxicol. Teratol 61, 36–46. [PubMed: 28286177]

Alshelh Z, Albrecht DS, Bergan C, Akeju O, Clauw DJ, Conboy L, Edwards RR, Kim M, Lee YC, Protsenko E, Napadow V, Sullivan K, Loggia ML, 2020 In-vivo imaging of neuroinflammation in veterans with Gulf War Illness. Brain Behav. Immun 1591 (19), 31334 pii: S0889.

Banks CN, Lein PJ, 2012 A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology 33 (3), 575–584. [PubMed: 22342984]

Barnes DE, Byers AL, Gardner RC, Seal KH, Boscardin WJ, Yaffe K, 2018 Association of mild traumatic brain injury with and without loss of consciousness with dementia in US military veterans. JAMA Neurol. 75 (9), 1055–1061. [PubMed: 29801145]

Belgrad J, Dutta DJ, Bromley-Coolidge S, Kelly KA, Michalovicz LT, Sullivan KA, O'Callaghan JP, Fields RD, 2019 Oligodendrocyte involvement in Gulf War Illness. Glia 67 (11), 2107–2124. [PubMed: 31339622]

Benjamini Y, Hochberg Y, 1995 Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc.: Ser. B (Methodol.) 57 (1), 289–300.

Bramlett HM, Dietrich WD, 2002 Quantitative changes in white and gray matter 1 year following traumatic brain injuries in rats. Acta Neuropathol. 103, 607–614. [PubMed: 12012093]

Broderick G, Kreitz A, Fuite J, Fletcher MA, Vernon SD, Klimas N, 2011 A pilot study of immune network remodeling under challenge in Gulf War Illness. Brain Behav. Immun 25 (2), 302–313. [PubMed: 20955779]

Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ, 1989 The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213. [PubMed: 2748771]

Chao LL, Rothlind JC, Cardenas VA, Meyerhoff DJ, Weiner MW, 2010 Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. NeuroToxicology 31 (5), 493–501. [PubMed: 20580739]

Chao LL, Abadjian L, Hlavin J, Meyerhoff DJ, Weiner MW, 2011 Effects of low-level sarin and cyclosarin exposure and Gulf War illness on brain structure and function: a study at 4T. NeuroToxicology 32, 814–822. [PubMed: 21741405]

Chao LL, Zhang Y, Buckley S, 2015 Effects of low-level sarin and cyclosarin exposure on white matter integrity in Gulf War Veterans. NeuroToxicology 48, 239–248. [PubMed: 25929683]

Chao LL, 2018 The relationship between traumatic brain injury and rates of chronic symptomatic illness in 202 Gulf War veterans. Mil. Med 183 (11–12), e571–e579. [PubMed: 29788089]

Colgan N, Siow B, O'Callaghan JM, Harrison IF, Wells JA, Holmes HE, Ismail O, Richardson S, Alexander DC, Collins EC, Fisher EM, Johnson R, Schwarz AJ, Ahmed Z, O'Neill MJ, Murray TK, Zhang H, Lythgoe MF, 2016 Application of neurite orientation dispersion and density imaging (NODDI) to a tau pathology model of Alzheimer's disease. NeuroImage 125, 739–744. [PubMed: 26505297]

Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ, 2006 An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. NeuroImage 31 (3), 968–980. [PubMed: 16530430]

Fischl B, 2012 FreeSurfer. NeuroImage 62 (2), 774–781. [PubMed: 22248573]

Fletcher MA, Zeng XR, Barnes Z, Levis S, Klimas NG, 2009 Plasma cytokines in women with chronic fatigue syndrome. J. Transl. Med 7, 96. [PubMed: 19909538]

Fukutomi H, Glasser MF, Zhang H, Autio JA, Coalson TS, Okada T, Togashi K, Van Essen DC, Hayashi T, 2018 Neurite imaging reveals microstructural variations in human cerebral cortical gray matter. NeuroImage 182 (15), 488–499. [PubMed: 29448073]

Gade DM, Wenger JB, 2011 Combat exposure and mental health: the long-term effects among US Vietnam and Gulf War veterans. Health Econ. 20, 401–416. [PubMed: 20336640]

Glasser MF, Goyal MS, Preuss TM, Raichle ME, Van Essen DC, 2014 Trends and properties of human cerebral cortex: Correlations with cortical myelin content. NeuroImage 93 (part. 2), 165–175. [PubMed: 23567887]

Grussu F, Schneider T, Tur C, Yates RL, Tachrount M, Ianus A, Yiannakas MC, Newcombe J, Zhang H, Alexander DC, DeLuca GC, Gandini Wheeler-Kingshott CAM, 2017 Neurite dispersion: a new marker of multiple sclerosis spinal cord pathology? Ann. Clin. Transl. Neurol 4 (9), 663–679. [PubMed: 28904988]

Golomb BA, 2008 Acetylcholinesterase inhibitors and Gulf War illnesses. Proc. Natl. Acad. Sci 105 (11), 4295–4300. [PubMed: 18332428]

Groppe DM, Urbach TP, Kutas M, 2014 Mass univariate analysis of event-related brain potentials/fields I: a critical tutorial review. Psychophysiology 48 (12), 1711–1725.

Gulani V, Webb A, Duncan I, Lauterbur P, 2001 Apparent diffusion tensor measurements in myelin-deficient rat spinal cords. Magn. Reson. Med 45, 191–195. [PubMed: 11180424]

Gur RC, Mozley PD, Resnick SM, Gottlieb GL, Kohn M, Zimmerman R, Herman G, Atlas S, Grossman R, Berretta D, 1991 Gender differences in age effect on brain atrophy measured by magnetic resonance imaging. PNAS 88 (7), 2845–2849. [PubMed: 2011592]

Hawari FI, Rouhani FN, Cui X, Yu ZX, Buckley C, Kaler M, Levine SJ, 2004 Release of full-length 55-kDa TNF receptor 1 in exosome-like vesicles: a mechanism for generation of soluble cytokine receptors. PNAS 101 (5), 1297–1302. [PubMed: 14745008]

Heaton KJ, Palumbo CL, Proctor SP, Killiany RJ, Yurgelun-Todd DA, White RF, 2007 Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. NeuroToxicology 28, 761–769. [PubMed: 17485118]

Hoge CW, McGurk D, Thomas JL, Cox AL, Engel CC, Castro CA, 2008 Mild traumatic brain injury in U.S. soldiers returning from Iraq. New England J. Med 358, 20.

Janulewicz P, Krengel M, Quinn E, Heeren T, Toomey R, Killiany R, Zundel C, Ajama J, O'Callaghan J, Steele L, Klimas N, Sullivan K, 2018 The multiple hit hypothesis for Gulf War illness: self-reported chemical/biological weapons exposure and mild traumatic brain injury. Brain Sci. 8 (11) pii: E198. [PubMed: 30428552]

Janulewicz PA, Seth RK, Carlson JM, Ajama J, Quinn E, Heeren T, Klimas N, Lasley SM, Horner RD, Sullivan K, Chatterjee S, 2019 The gut-microbiome in Gulf War veterans: A preliminary report. Int. J. Environ. Res. Public Health 16, 3571.

Jaundoo R, Bohmann J, Gutierrez GE, Klimas N, Broderick G, Craddock TJA, 2018 Using a consensus docking approach to predict adverse drug reactions in combination drug therapies for Gulf War illness. Int. J. Mol. Sci 19, 3355.

Jeffrey MG, Krengel M, Kibler JL, Zundel C, Klimas NG, Sullivan K, Craddock TJA, 2019 Neuropsychological findings in Gulf War illness: a review. Front. Psychol 10, 2088. [PubMed: 31616335]

Jenkinson M, Beckmann CF, Behrens TEJ, Woolrich MW, Smith SM, 2012 FSL. NeuroImage 62, 782–790. [PubMed: 21979382]

Johnson GA, Calabrese E, Little PB, Hedlund L, Qi Y, Badea A, 2014 Quantitative mapping of trimethyltin injury in the rat brain using magnetic resonance histology. Neurotoxicology 42, 12–23. [PubMed: 24631313]

Khaiboullina SF, DeMeirleir KL, Rawat S, Berk GS, Gaynor-Berk RS, Mijatovic T, Blatt N, Rizvanov AA, Young SG, Lombardi VC, 2014 Cytokine expression provides clues to the pathophysiology of Gulf War illness and myalgic encephalomyelitis. Cytokine 72 (1), 1–8. [PubMed: 25514671]

Kimura Y, Sato N, Ota M, Shigemoto Y, Morimoto E, Enokizono M, Matsuda H, Shin I, Amano K, Ono H, Sato W, Yamamura T, 2019 Brain abnormalities in myalgic encephalomyelitis/chronic fatigue syndrome: evaluation by diffusional kurtosis imaging and neurite diffusional kurtosis imaging and neurite orientation dispersion and density imaging. J. Magn. Reson. Imaging 49, 818–824. [PubMed: 30430664]

Koo BB, Michalovicz LT, Calderazzo S, Kelly KA, Sullivan K, Killiany RJ, O'Callaghan JP, 2018 Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. Brain Behavior, and Immunity 67, 42–46.

Kumar A, Loane DJ, 2012 Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav. Immun 26, 1191–1201. [PubMed: 22728326]

Locker AR, Michalovicz LT, Kelly KA, Miller JV, Miller DB, O'Callaghan JP, 2017 Corticosterone primes the neuroinflammatory response to Gulf War Illness-relevant organophosphates independently of acetylcholinesterase inhibition. J. Neurochem 142 (3), 444–455. [PubMed: 28500787]

McCunn P, Gilbert KM, Zeman P, Li AX, Strong MJ, Khan AR, Bartha R, 2019 Reproducibility of neurite orientation dispersion and density imaging (NODDI) in rats at 9.4 Tesla. PLoS ONE 14 (4), e0215974.

Melzack R, 1975 The McGill Pain Questionnaire: Major properties and scoring methods. PAIN 1 (3), 277–299. [PubMed: 1235985]

Michalovicz LT, Locker AR, Kelly KA, Miller JA, Barnes Z, Fletcher MA, Miller DB, Klimas NG, Morris M, Lasley SM, O'Callaghan JP, 2019 Corticosterone and pyridostigmine/DEET exposure attenuate peripheral cytokine expression: Supporting a dominant role for neuroinflammation in a mouse model of Gulf War Illness. NeuroToxicology 70, 26–32. [PubMed: 30339781]

National Academies of Sciences, Engineering, and Medicine. (2016). Gulf War and Health: Volume 10: Update of Health Effects of Serving in the Gulf War, 2016. Washington, DC: The National Academies Press.

Miller JV, LeBouf RF, Kelly KA, Michalovicz LT, Ranpara A, Locker AR, Miller DB, O'Callaghan JP, 2018 The neuroinflammatory phenotype in a mouse model of Gulf War illness is unrelated to brain regional levels of acetylcholine as measured by quantitative HILIC-UPLC-MS/MS. Toxicol. Sci 165 (2), 302–313. [PubMed: 29846716]

Milligan E, Watkins L, 2009 Pathological and protective roles of glia in chronic pain. Nat. Rev. Neurosci 10, 23–26. [PubMed: 19096368]

Naughton SX, Hernandez CM, Beck WD, Poddar I, Yanasak N, Lin PC, Terry AV Jr., 2018 Repeated exposures to diisopropylfluorophosphate result in structural disruptions of myelinated axons and persistent impairments of axonal transport in the brains of rats. Toxicology 406–407, 92–103.

O'Callaghan JP, Kelly KA, Locker AR, Miller DB, Lasley SM, 2015 Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. J. Neurochem 133, 708–721. [PubMed: 25753028]

O'Callaghan JP, Miller DB, 2019 Neuroinflammation disorders exacerbated by environmental stressors. Metabolism 100S, 153951.

O'Donovan A, Chao LL, Paulson J, Samuelson KW, Shigenaga JK, Grunfeld C, Weiner MW, Neylan TC, 2015 Altered inflammatory activity associated with reduced hippocampal volume and more severe posttraumatic stress symptoms in Gulf War veterans. Psychoneuroendocrinology 51, 557–566. [PubMed: 25465168]

Parkitny L, Middleton S, Baker K, Younger J, 2015 Evidence for abnormal cytokine expression in Gulf War Illness: a preliminary analysis of daily immune monitoring data. BMC Immunol. 16, 57. [PubMed: 26420016]

Proctor SP, Heaton KJ, Heeren T, White RF, 2006 Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. NeuroToxicology 27 (6), 931–939. [PubMed: 16982099]

Proctor SP, Heeren T, White RF, Wolfe J, Borgos MS, Davis JD, Pepper L, Clapp R, Sutker PB, Vasterling JJ, Oznoff D, 1998 Health status of Persian Gulf War veterans: self-reported symptoms, environmental exposures and the effect of stress. Int. J. Epidemiol 27 (6), 1000–1010. [PubMed: 10024195]

Puri BK, Jakeman PM, Agour M, Gunatilake KDR, Fernando KAC, Gurusinghe AI, Treasaden IH, Waldman AD, Gishen P, 2012 Regional grey and white matter volumetric changes in myalgic encephalomyelitis (chronic fatigue syndrome): a voxel-based morphometry 3T MRI study. British J. Radiol 85, e270–e273.

Raivich G, Jones LL, Werner A, Blüthmann H, Doetschmann T, Kreutzberg GW, 1999 Molecular signals for glial activation: Pro- and anti-inflammatory cytokines in the injured brain. Acta Neurochir. Suppl 73, 21–30. [PubMed: 10494337]

Rao AN, Patil A, Brodnik ZD, Qiang L, España RA, Sullivan KA, Black MM, Baas PW, 2017 Pharmacologically increasing microtubule acetylation corrects stress-exacerbated effects of organophosphates on neurons. Traffic 18 (7), 433–441. [PubMed: 28471062]

Rathbone AT, Tharmaradinam S, Jiang S, Rathbone MP, Kumbhare DA, 2015 A review of the neuro- and systemic inflammatory responses in post concussion symptoms: Introduction of the "post-inflammatory brain syndrome". PIBS Brain Behav. Immun 46, 1–16. [PubMed: 25736063]

Rayhan RU, Stevens BW, Timbol CR, Adewuyi O, Walitt B, VanMeter JW, Baraniuk JN, 2013 Increased brain white matter axial diffusivity associated with fatigue, pain, and hyperalgesia in Gulf War illness. PLoS ONE 8 (3), e58493.

Robbins CA, Daneshevar DH, Picano JD, Gevett BE, Baugh CM, Riley DO, Nowinski CJ, McKee AC, Cantu RC, Stern RA, 2014 Self-reported concussion history: impact of providing a definition of concussion. Open Access J. Sports Med 7, 99–103.

Seichepine DR, Stamm JM, Daneshvar DH, Riley DO, Baugh CM, Gavett BE, Tripodis Y, Martin B, Chaisson C, McKee AC, Cantu RA, Nowinski CJ, Stern RA, 2013 Profile of self-reported problems with executive functioning in college and professional football players. J. Neurotrauma 30, 1299–1304. [PubMed: 23421745]

Smets EMA, Garssen B, Bonke B, De Haes JCJM, 1995 The Multidimensional Fatigue Inventory (MFI) Psychometric Qualities of an Instrument to Assess Fatigue. J. Psychosom. Res 39 (5), 315–325. [PubMed: 7636775]

Smith DH, Johnson VE, Stewart W, 2013 Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nature Rev. Neurol 9 (4), 211–221. [PubMed: 23458973]

Spradling KD, Lumley LA, Robison CL, Meyerhoff JL, Dillman JF, 2011 Transcriptional responses of the nerve agent-sensitive brain regions amygdala, hippocampus, piriform cortex, septum, and thalamus following exposure to the organophosphonate anticholinesterase sarin. J. Neuroinflammation 8, 84. [PubMed: 21777430]

Steele L, 2000 Prevalence and patterns of Gulf War illness in kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am. J. Epidemiol 152 (10), 922–1002.

Sullivan K, Krengel M, Bradford W, Stone C, Thompson TA, Heeren T, White RF, 2017 Neuropsychological functioning in military pesticide applicators from the Gulf War: effects on information processing speed, attention and visual memory. Neurotoxicol. Teratol 65, 1–13. [PubMed: 29126934]

Sullivan K, Krengel M, Proctor SP, Devine S, Heeren T, White RF, 2003 Cognitive functioning in treatment-seeking Gulf War veterans: pyridostigmine bromide use and PTSD. J. Psychopathol. Behav. Assess 25, 95–103.

Tang LW, Zheng H, Chen L, Zhou SY, Huang WJ, Li Y, Wu X, 2015 Gray matter volumes in patients with chronic fatigue syndrome. pii:380615. Evidence-Based Complementary Alternative Med

Toomey R, Alpern R, Vasterling JJ, Baker DG, Reda DJ, Lyons MJ, Henderson WG, Kang HK, Eisen SA, Murphy FM, 2009 Neuropsychological functioning of U.S. Gulf War veterans 10 years after the war. J. Int. Neuropsychological Soc 15 (5), 717–729.

Vynorius KC, Paquin AM, Seichepine DR, 2016 Lifetime multiple mild traumatic brain injuries are associated with cognitive and mood symptoms in young healthy college students. Front. Neurol 7, 188. [PubMed: 27843436]

Tuch DS, 2004 Q-ball Imaging. Magn. Reson. Med 52 (6), 1358–1372. [PubMed: 15562495]

Whistler T, Fletcher MA, Lonergan W, Zeng XR, Lin JM, LaPerriere A, Vernon SD, Klimas NG, 2009 Impaired immune function in Gulf War Illness. BMC Med. Genomics 2, 12.

White RF, Steele L, O'Callaghan JP, Sullivan K, Binns JH, Golomb BA, Bloom FE, Bunker JA, Crawford F, Graves JC, Hardie A, Klimas N, Knox M, Meggs WJ, Melling J, Philbert MA, Grashow R, 2016 Recent research on Gulf War Illness and other health problems in veterans of the 1991 Gulf War: Effects of toxicant exposures during deployment. Cortex 74, 449–475. [PubMed: 26493934]

Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE, 2014 Permutation inference for the general linear model. NeuroImage 92, 381–397. [PubMed: 24530839]

Xanthoulea S, Pasparakis M, Kousteni S, Brakebusch C, Wallach D, Bauer J, Lassmann H, Kollias G, 2004 Tumor Necrosis Factor (TNF) receptor shedding controls thresholds of innate immune activation that balance opposing TNF functions in infectious and inflammatory diseases. J. Exp. Med 200 (3), 367–376. [PubMed: 15289505]

Yee MK, Seichepine DR, Janulewicz PA, Sullivan KA, Proctor SP, Krengel MH, 2016 Self-reported traumatic brain injury, health and rate of chronic multisymptom illness in veterans from the 1990–1991 Gulf War. J. Head Trauma Rehabilitation 31 (5), 320–328.

Yee MK, Janulewicz PA, Seichepine DR, Sullivan KA, Proctor SP, Krengel MH, 2017 Multiple mild traumatic brain injuries are associated with increased rates of health symptoms and Gulf War illness in a Cohort of 1990–1991 Gulf War Veterans. Brain Sciences 7, 79.

Yeh FC, Wedeen VJ, Tseng WY, 2010 Generalized q-Sampling Imaging. IEEE Trans. Med. Imaging 29 (9), 1626–1635. [PubMed: 20304721]

Yeh FC, Liu L, Hitchens TK, Wu YL, 2017 Mapping immune cell infiltration using restricted diffusion MRI. Magn. Reson. Med 77, 603–612. [PubMed: 26843524]

Yendiki A, Panneck P, Srinivasan P, Stevens A, Zöllei L, Augustinack J, Wang R, Salat D, Ehrlich S, Behrens T, Jbabdi S, Gollub R, Fischl B (2011). Automated probabilistic reconstruction of white-

matter pathways in health and disease using an atlas of the underlying anatomy. frontiers in neuroinformatics, 5.

Zelová H, Hošek J, 2013 TNF-a signaling and inflammation: interactions between old acquaintances. Inflamm. Res 62, 641–651. [PubMed: 23685857]

Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC, 2012 NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. NeuroImage 61, 1000–1016. [PubMed: 22484410]

Zhuo J, Xu S, Proctor JL, Mullins RJ, Simon JZ, Fiskum G, Gullapalli RP, 2012 Diffusion kurtosis as an in vivo imaging marker for reactive astrogliosis in traumatic brain injury. Neuroimage 59 (1), 467–477. [PubMed: 21835250]

Zundel CG, Krengel MH, Heeren T, Yee MK, Grasso CM, Janulewicz Lloyd PA, Coughlin SS, Sullivan K, 2019 Rates of Chronic Medical Conditions in 1991 Gulf War Veterans Compared to the General Population. Int. J. Environ. Res. Public Health 16 (6) pii: E949. [PubMed: 30884809]

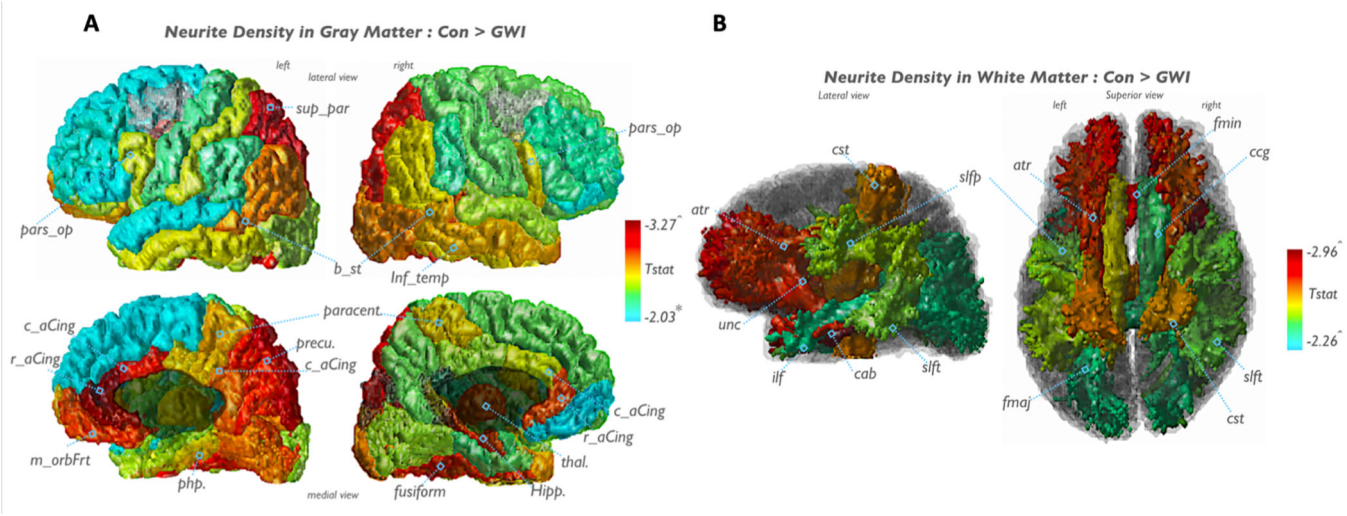

Fig. 1.

ND feature mapping of whole group WM and GM analyses highlights group effects in widespread regions, most significantly seen in frontal white matter tracts and subcortical limbic regions. Fmaj = corpus callosum forceps major, fmin = corpus callosum forceps minor, atr = anterior thalamic radiations, cab = cingulum-angular bundle, ccg = cingulate gyrus bundle, cst = corticospinal tract, ilf = inferior longitudinal fasciculus, slfp = superior longitudinal fasciculus parietal, slft = superior longitudinal fasciculus temporal, unc = uncinate fasciculus, pars_op = pars opercularis, sup_par = superior parietal, b_st = banks of superior temporal sulcus, inf_temp = inferior temporal, c_aCing = caudal anterior cingulate, r_aCing = rostral anterior cingulate, m_orbFrt = medial orbitofrontal, php = parahippocampal, hipp = hippocampus, thal = thalamus proper, precu = precuneus, paracent = paracentral. * $p < 0.05$, ^ FDR_adj_$p < 0.05$.

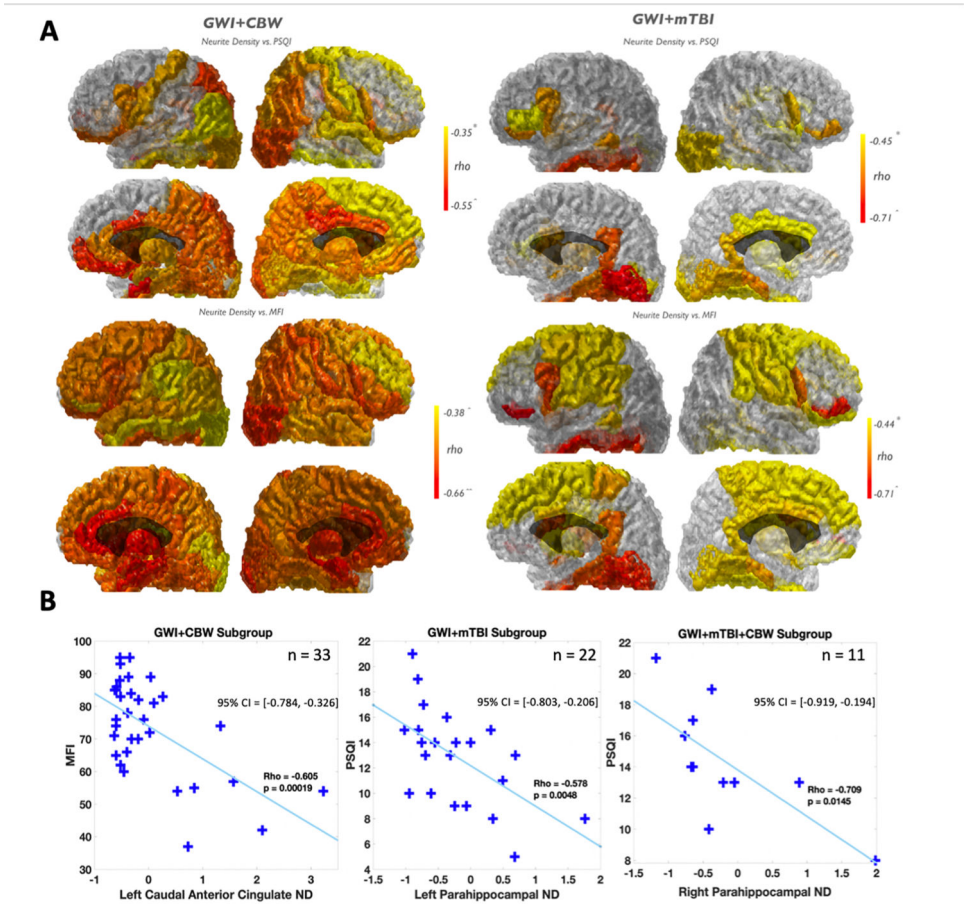

Fig. 2.

Self-reported symptoms correlation mapping in GWI subjects exposed to chemical or biological warfare agents or mTBI. Regions with significant correlation between ND and PSQI (A, left upper) or MFI (A, left lower) in GW veterans with chemical/biological warfare agent exposures are rendered based on significance levels. Regions with significant correlation between ND and PSQI (A, right upper) or MFI (A, right lower) in GW veterans with mTBI exposure are rendered based on significance levels. Panel B shows data distribution patterns of ND and PSQI (B, middle and right) or MFI (B, left) scores in representative regions within each subgroup. Some subjects did not have available PSQI data, therefore, the number of subjects (n) used for subgroup correlation is indicated in the figure and 95% CIs are provided. * $p < 0.05$,^ FDR_adj_$p < 0.05$, ⩓ FDR_adj_$p < 0.01$.

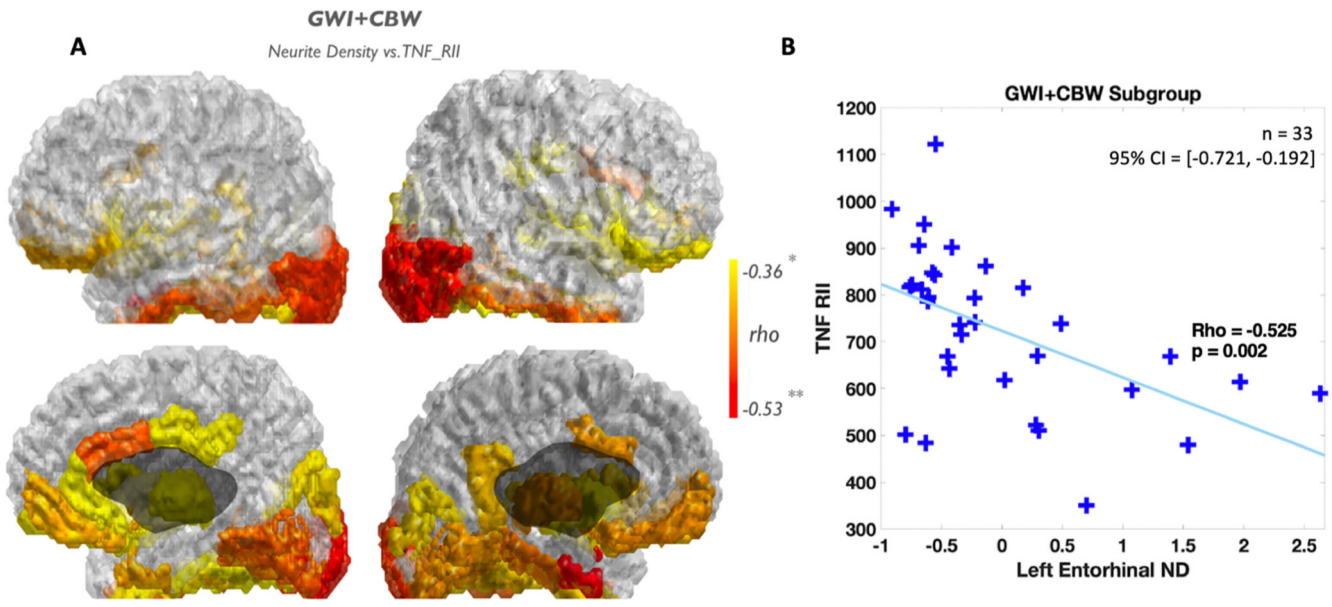

Fig. 3.

Blood cytokine correlation mapping in GWI subjects exposed to chemical and biological warfare agents. Regions with significant correlation between ND and TNF_RII (A) are rendered based on significance levels. Panel B shows data distribution patterns of ND and TNF_RII levels in the representative region within the GWI + CBW subgroup. * $p < 0.05$, ** $p < 0.01$.

Table 1

Demographic and self-reported exposure to risk factors information for GWI case and control subjects.

	GW Control	GWI Case
N	16	75
Age (years)	53.85	52.07
Gender (F/M)	1/15	16/59
Exposure to risk factors during war (% exposed)		
Mild traumatic brain injury (mTBI)	0%	30.67%
Chemical/Biological warfare agents (CBW)	12.50%	44%
mTBI + Chem/Bio warfare agents (mTBI + CBW)	0%	16%