AWARD NUMBER: W81XWH-17-1-0440

TITLE: Computer aided decoding of brain-immune interactions in Gulf War Illness (GWI): a joint embedding on brain connectomic and immunogenetic markers

PRINCIPAL INVESTIGATOR: BANG-BON KOO, Ph.D.

CONTRACTING ORGANIZATION: Boston University School of Medicine, Boston, MA

REPORT DATE: December 2021

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2021	Final report	01Sep2017-31Aug2021

4. TITLE AND SUBTITLE
Computer aided decoding of brain-immune interactions in Gulf War Illness (GWI): a joint embedding on brain connectomic and immunogenetic markers.

5a. CONTRACT NUMBER
W81XWH-17-1-0440

5b. GRANT NUMBER
50206023

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bang-Bon Koo
E-Mail: bbkoo@bu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Trustees of Boston University
85 E NEWTON ST M 921
BOSTON MA 02118-2340

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
Gulf War Illness, Neuroimaging, Immune markers, Cognitive markers, Machine learning

14. ABSTRACT

Brain-immune interaction is a key factor to understanding the origin of complex symptoms in GWI. This project was designed to study the brain-immune interactions by utilizing a novel computer aided decoding scheme. Throughout 4 years of project period, we developed an image processing pipeline and computational frameworks for analyzing multi-modal magnetic resonance imaging (MRI) data collected from the Gulf War Illness Consortium (GWIC). Based on this, we have identified potential diagnostic markers for GWI from an abundant amount of imaging markers and also built a machine learning (ML) framework for classifying GWI in a single-subject level. Selected MRI biomarkers were cross-compared to following markers: cognitive, blood immune, and central immune markers. We also examined exposure/symptom-specific MRI markers. ML classifiers were tested across a single modality MRI, multi-modality MRI or MRI markers combined with others (e.g., cognitive or blood immune markers) to identify the best performing models for diagnosing GWI. From these works, key findings include, 1) Diffusion MRI markers provided best performing ML model, 2) Joint embedding of MRI and non-imaging markers help enhancing the ML performance, 3) More clear brain immune interactions found in exposure-specific subgroups, 4) There are variant MRI markers associated with different symptoms. We published these findings in scientific journals and the results were also presented in scientific conferences. Findings (i.e., features and statistical results) and techniques developed in this project were also packed into a software package as planned and ready to be shared to the GWI research community through collaborations to support building more practical and robust technical as well as clinical solutions for GWI.

15. SUBJECT TERMS. Gulf War Illness, Neuroimaging, Immune markers, Cognitive markers, Machine learning

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	139	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)

TABLE OF CONTENTS

1. INTRODUCTION:

At least 25-30% of the nearly 700,000 U.S. veterans who served in the 1991 Gulf War continue to suffer from a complex, multisystem disorder called Gulf War Illness (GWI). Converging evidence suggests that a clear understanding of brain-immune interactions can be key to understanding the origin of these symptoms. In addition, the identification of immune activation, brain imaging, and genetic biomarkers has been recommended by GWI advisory committees as research targets. To date, multiple studies have suggested various candidate markers in different biological domains, however, they were in isolation and lacked information on how to unite these markers to better represent the brain-immune interactions underlying GWI. The central hypothesis of this new investigator project is that incorporating joint distributional information across biomarkers from different biological domains can provide more meaningful insight into GWI etiology than can be obtained from a single marker or a simple concatenation of different markers within a single biological domain. This study was planned to take various promising biological (i.e., brain imaging and immune) measures of GWI in collaboration with a large GWI Consortium (GWIC) study (GW120037, PI: Sullivan) and designed for combining the enormous amount of GWIC biomarker data into a cutting-edge machine-learning computational framework to better represent and understand the brain-immune interactions underlying GWI. Throughout the entire project years, building and assurance testing of the proposed analysis framework was performed based on the GWIC data. Combining this enormous amount of biomarker data into a cohesive computational model allows for the development of biomarkers that represent GWI and most importantly to map these markers in individual subject space rather than only looking at information at the group level. The impact of identifying validated and replicated biomarkers of GWI and identifying how predictive each marker is of the other can be an invaluable resource in moving the field forward and for reliably using those biomarkers to predict future treatment trial efficacy at the group and individual veteran level.

2. KEYWORDS:

Gulf War Illness (GWI), White matter (WM) integrity, Gray matter (GM) microstructure, Brain mapping, Morphometry, Neuroinflammation, Magnetic Resonance Imaging (MRI), Cognitive test, Machine learning, Blood Cytokine, Exposures, Symptoms, Kansas criteria, CDC criteria, Database, Software.

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The central goal of this project is to build a comprehensive computational framework that incorporates joint distributional information across different biomarkers for decoding brain-immune interactions and providing a better illustration of GWI etiology over single modality biological measurements. Specific major goals of this project include: 1) Application of multimodal MRI processing pipeline and extract high-quality post-processed data, 2) Building unimodal classifiers on neuroimaging and neuroimmune marker data, 3) Building multi-modal classifiers and defining of key features, 4) Building multi-modality classifiers of sub-symptom clusters, 5) Present and publish the results, and 6) Building a software package.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major Activities

Throughout the entire project years, following major activities were performed aiming each specific major goal listed above.

a. MRI image processing pipeline: We built image processing pipelines for T1-structural and high dimensional diffusion MRI (hd-MRI) data. The first version of the pipeline includes following functions: 1) cortical surface modeling based on Freesurfer (Fischl, 2012) and defining in region of interest (ROI) in the brain, 2) extraction of morphometrical features including cortical thickness, cortical/sub-cortical volume, hippocampal and amygdala sub-volume, intra-cranial volume, 3) hd-MRI data preprocessing including distortion and motion correction, T1 to diffusion co-registration, 4) generalized q-space imaging (GQI) reconstruction for hd-MRI, which provides micro-diffusivity used in our previous study (Koo et al., 2018), generalized fractional anisotropy, major and minor diffusivity, 5) neurite density imaging (NDI) reconstruction in WM, 6) NDI for GM based on maximum likelihood estimation of model-fitting parameters, 7) diffusion tensor imaging reconstruction on b-1000 shell (DTI) and b-3000 shell

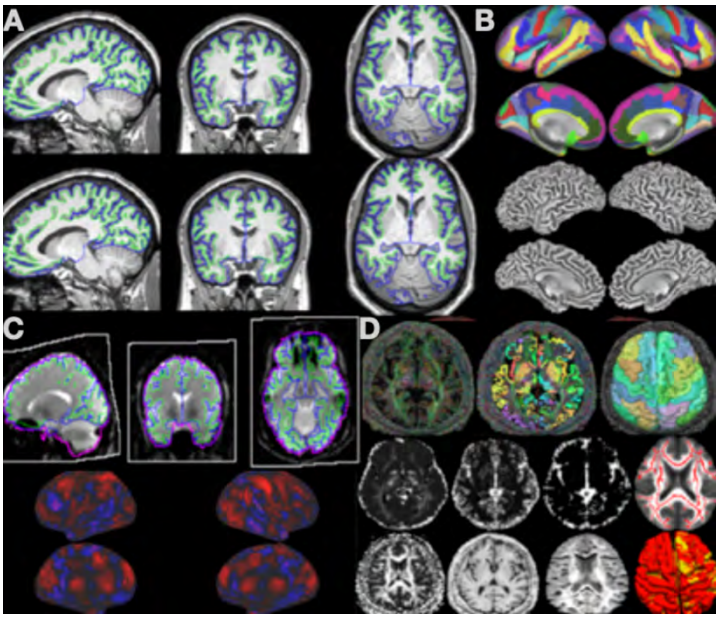


Figure 1. Image processing pipeline. Panel A: morphometrical processing and surface modeling. Panel B: Cortical parcellations using surface modeling. Panel C: Coregistration between structural and diffusion MRI. Panel D: Diffusion processing pipeline.

(HARDI-DTI), 8) WM fiber reconstructions (using cortical ROIs to reconstruct WM tracts), 9) mapping and extracting morphological and diffusion measures in each ROI.

Some features were continuously updated or added to this package throughout the project years. Firstly, WM fiber reconstructions were changed from ROI-to-ROI WM tracking method to defining major WM bundles using TRACULA (Yendiki et al., 2011). This was to reduce the number of features without losing critical information from the diffusion MRI. Formal method yields too many features compared to the sample size, which can cause overestimation problems on ML classification works. We also added additional features for extracting WM measures using tract-based spatial statistics approach TBSS, Smith et al., 2006). This extracts diffusion measures along the core WM pathway in each tract. Subjects with abnormal ventricles often can cause WM tract estimation errors in TRACULA. This additional feature supports fixing the problem. Secondly, we added other diffusion MRI modeling techniques. The pipeline currently offers free-water diffusion image reconstruction (Pasternak et al., 2009) and diffusion kurtosis image reconstruction (Zhuo et al., 2012). Recent publications based on diffusion MRI often use different diffusion modeling techniques, which makes it hard to integrate different findings. This was to give more degrees of freedom on processing diffusion MRI data and to give options for cross-compare results from different models. Lastly, we added morphological network measures based on structural MRI. Morphological network measures were previously used in other studies for detecting potential synchronized structural changes between cortical regions (Evans, 2013). This measure can be tested as an alternative marker when diffusion MRI is not available for the study.

b. Statistical analysis on MRI, immune, cognitive features: We performed statistical analyses on all extracted measures. Group differences between GWI cases and controls were first assessed on NDI, DTI, GQI, and morphological measures. We also performed group comparisons between exposure/symptom based GWI subgroups and controls. MRI markers were also compared with blood immune markers, symptom scores and cognitive functions, in both whole group and subgroup levels. We also performed correlation analysis between different MRI markers.

c. ML classification framework and benchmark tests: The initial ML framework was developed based on random decision forest (ref). RDF performs iterative partitioning of the multivariate feature space to identify decision boundaries that highlight differences between different groups. RDF also provides feature importance weights to each of the features selected for the classification and thereby allow us to identify which combinations of features among that we have provide the best sensitivity for the group comparisons. RDF was then updated to random decision forest based on rotating feature space method (CCF; Rainforth & Wood, 2009), are applied simultaneously for estimating maximum performance on the selected feature sets. In our benchmark tests, CCF performed better then RDF. We also added a reinforcement learning ML method (Guan et al., 2020). This method allows adaptive and efficient parameter optimization for finding best solutions on classification. Finally, CCF was also updated to CCF with synthetic oversampling method (SMOTE) to handle imbalanced grouping issues. Based on these ML classification algorithms, classification testing was targeted to 1) GWI cases vs. controls based on Kansas criteria, 2) GWI cases vs. controls based on CDC criteria, and 3) GWI case subgroups (based on exposures and health profiles) vs controls. In each classification targets, we first applied single modality features from either MRI (i.e., features from NDI, GQI, DTI, and morphometry) or blood cytokines. Then, combinations of different features (i.e., different imaging modality or imaging + non-imaging markers). For the first and second classification targets,

we combined MRI markers with either exposure conditions (e.g., pesticides) or health profiles (e.g., high blood pressure, depression, or post-traumatic stress disorder) for the classification attempt. Performance of the classifiers were cross-validated and key features were investigated from this work.

d. Disseminate study results: Three papers were published in scientific journals from PI's group. We have one paper taking 2nd round review in a scientific journal. We also have one paper ready for submission. Results were also shared through two domestic meetings, four international meetings, and one invited seminar. Some findings and techniques were shared to GWIC collaborators and resulted in two journal publications. We are now preparing one more manuscript for publication through this collaboration work.

e. Building software package: We started the software package in project year 3. This work was expanded to the NCE period and ready for sharing to the GWI research community through collaboration. Detailed descriptions on this software package can be found in the significant results or key outcomes section.

2) Specific objectives

In the project year 1, we aimed to acquire high quality post-processed data and neuroimaging classifiers. From the processed data, we also aimed to explore whether GWI is more associated with alterations in the local brain components especially in the microscopic structural integrity and connectivity in the white matter (WM).

In the project year 2, we continued to acquire high quality post-processed data and neuroimaging classifiers. We aimed to explore brain imaging fingerprints on GWI. Mass-univariate statistics on each of the brain imaging measures was our first analytic scheme to define key neurological markers from imaging data. We also tested different classification designs. Defining key features and cross validating different types of machine learning classification methods were the specific goals for this project year.

In the project year 3, we continued to add high quality post-processed data and develop neuroimaging classifiers. We specifically aimed to build a more efficient machine learning framework to define key neurological markers from imaging data to integrate different biomarkers from different biological domains (imaging to cytokine markers) to investigate meaningful insight into GWI etiology. In this project year, the research team

In the no-cost extension (NCE) period (year 4), we aimed to add more subject data from GWIC and finalize building the classifiers. We also planned to start testing and combining new imaging markers (glial PET and resting fMRI) extracted from the existing GWIC to expand imaging feature domain to cover brain functional aspects. We also aimed to study associations between new and existing markers. Lastly, in this NCE period, we planned to finalize the building software package containing key results and techniques achieved from this project.

3) Significant results or key outcomes, including major findings, developments, or conclusions

a. High-dimensional diffusion MRI (hd-MRI) markers for GWI:

- Validation of hd-MRI in animal models: In our first animal study, we had tested micro-diffusion imaging on sarin-surrogate (diisopropyl fluorophosphate DFP) and/or corticosterone (CORT) treated rat models to assess the GW-relevant neurotoxicant and physical stressors of deployment (Koo et al., 2018). We had applied 500-micron image resolution using our 4.7T Bruker MRI system. The use of the rat model allowed us to apply a combination of standard histological methods and advanced immunohistochemical methods to optimally prepare brain tissue from the same MRI assessed brains. We have confirmed that the micro-diffusion imaging successfully differentiated CORT stressor responses in the brain in different diffusion components (Figure 2A). Rats exposed to either the sarin surrogate DFP and/or CORT had significant increase in micro-diffusivity (based on GQI) especially in the hypothalamus and hippocampus, whereas the fast diffusion imaging did not show significant differences. Micro-diffusion imaging also revealed different spatial patterns in 3 different treatment models (CORT, DFP, CORT+DFP). Cortical regions with lowered diffusivities were correlated with tissue assessment results. We expanded this work to study chronic models. In the chronic model, group differences were maintained in CORT and CORT+DFP groups (Figure 2B). We also confirmed the layering effect (i.e., highest signal observed from exposure to CORT, DFP plus LPS) from the microstructural diffusion markers (GQI and NDI).

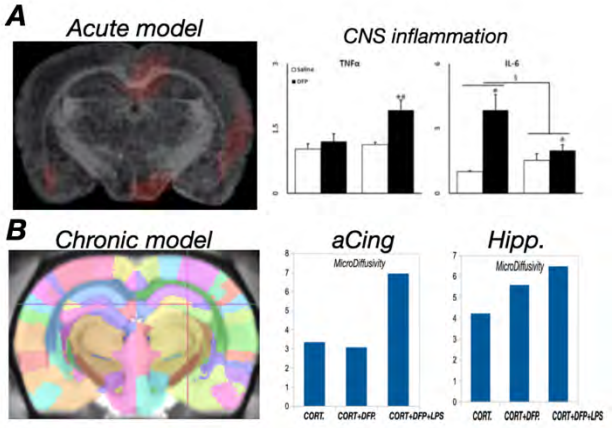


Figure 2. hd-MRI in animal models. Panel A shows imaging results in acute rat model. Red marks indicate the regions showing difference between groups. Panel B shows imaging results in chronic model. We applied automatic labeling technique (left side). Bar graphs showing micron diffusivity in anterior cingulate and hippocampus in 3 groups (cort, cort+DFP, cort+DFP+LPS.

- hd-MRI and translocator protein positron emission tomography (TSPO-PET): hd-MRI markers were also compared with TSPO-PET. TSPO-PET can highlight activated glial cells in the brain. NDI markers, such as OD and isotropic freewater fraction (IsoVF), showed overlapping patterns in the cingulate cortex and other regions when compared separately for positron emission tomography (PET) scans using the translocator protein (TSPO) (Alshelh et al., 2020) (Figure 3. 3D plot). Statistically significant correlations were confirmed in these two different imaging methods

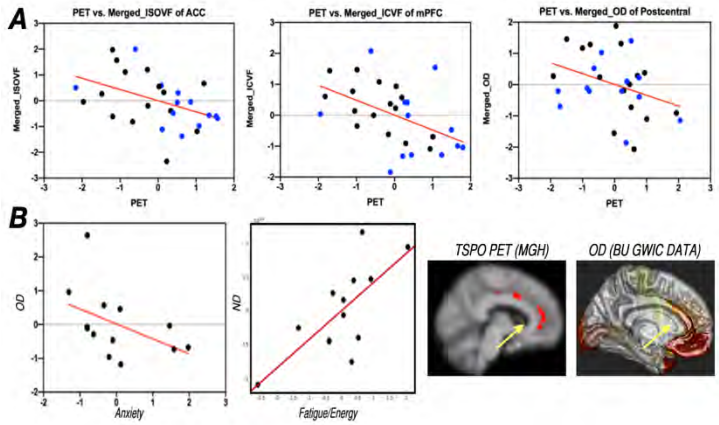


Figure 3. Comparisons between hd-MRI and TSPO-PET. NDI features significantly correlated with TSPO pet signal. Panel A: PET vs. NDI in anterior cingulate (left), medial prefrontal (middle), and post-central (right). Panel B: NDI additionally explained the illness symptoms. Left graph: OD vs Anxiety. Right graph: ND vs Fatigue/Energy (high score better condition). Right side 3D renderings show activated signal in ACC (left) and lowered OD in ACC.

(Figure 3A). Moreover, hd-MRI markers additionally explained GWI veteran's illness symptoms (e.g., fatigue, pain, anxiety) (Figure 3B). Both animal model findings and TSPO-PET results, support that microscopic diffusivity can fingerprint brain-immune interactions in GWI.

- hd-MRI in WM: NDI mapping in the major WM fibers consistently showed signs of weakened WM integrity (lowered neurite density). Overall pattern was consistent to our previous findings. All major WM tracts showed significant differences in ND and OD for GWI cases compared to controls. In neurite density (ND) measures, the greatest significant group differences between GWI cases and controls were seen in the bilateral corticospinal tracts followed by callosal tracts and others. Compared to our first analysis based on 91 subject data, only CST results become more significant ($t=3.38$, $p<0.001$ corrected). Other tracts revealed patterns consistent to our previous findings (Cheng et al., 2020).

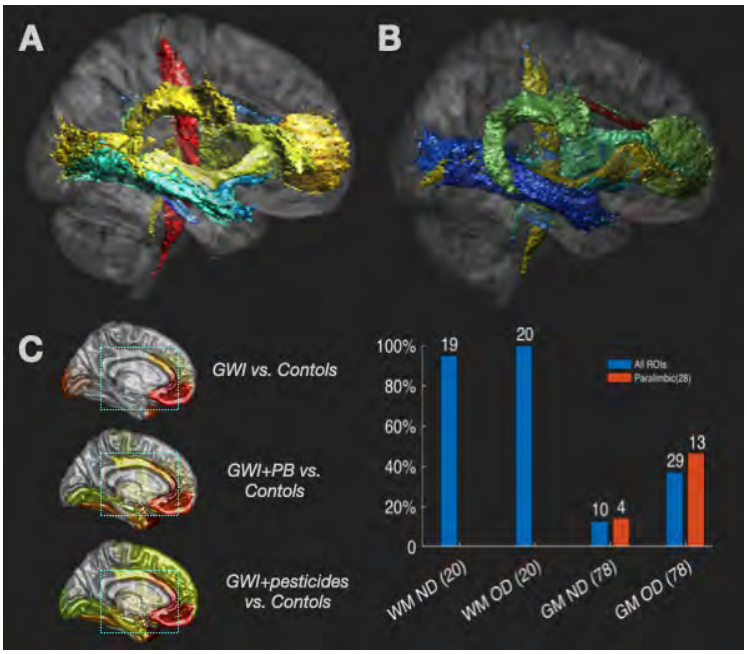


Figure 4. NDI results from GWIC data. Panel A shows group differences in GWI case and controls in WM ND. Panel B shows WM OD results. Panel C shows subgroup analysis in GM ND. Right bar graph shows counts of significant features in each feature domain.

Both WN ND (figure 4A) and orientation dispersion (OD, figure 4B) measures showed widespread differences. Nineteen out of twenty WM tracts were significantly different in ND, while all twenty WM tracts were significantly different between GWI cases and controls (Figure 4 graph).

- hd-MRI in GM: NDI mapping in GM also revealed significant group differences between GWI cases and controls. Most significant group differences were observed in anterior cingulate cortex (ACC) bilaterally. As we discussed previously, this result coincides with glial PET findings. OD in ACC was also significantly correlated with Kansas fatigue and pain scores. Other limbic and paralimbic regions (e.g., parahippocampal gyrus, isthmus cingulate, precuneus, and fusiform) showed near-significant group differences in uncorrected $p<0.05$ level. Difference patterns were more significant in GWI exposure specific subgroup analysis (e.g., GWI + Pyridostigmine Bromide vs. controls, GWI + pesticides vs. controls) (figure 4C) and GWI health profile subgroupings (e.g., GWI + high blood pressure, depression or post-traumatic stress disorder). However, not all the exposure or health profile subgroups showed significant differences. For example, GWI case groups who reported exposure to destroyed enemy vehicles did not show any significant group differences in GM NDI measures. This heterogeneity suggests that different pathophysiology may sit in this complex multi-symptom illness. In addition, compared to WM NDI measures, GM NDI measures showed more clear associations with blood cytokine and symptom scores.

- DTI findings: Diffusion MRI data in two different diffusion encodings, b=1,000 s/mm^2 (low-b) and 3,000 s/mm^2 (high-b) were extracted separately from hd-MRI. DTI reconstruction was applied to each

of diffusion encodings and used for group comparison between GWI cases and controls. This was to assess whether consistent patterns can be captured from different types of diffusion modeling and diffusion encoding strengths. In GWI cases vs. controls, high-b DTI showed group differences in anterior and posterior corpus callosal tracts, bilateral inferior longitudinal fasciculus (ILF), right inferior fronto-occipital fasciculus (IFOF), and right CST consistent to NDI results. However, there were no significant group differences shown in low-b DTI, which suggests that microscale diffusivity measures are more sensitive to detect GWI specific change patterns. Similar to what we confirmed in animal models, this also suggests that neuroinflammatory response accompanies changes in sub-neuronal (e.g., glial activation and synaptic integrity changes) scale rather than significant neuronal cell loss. It is also important to note that GW veterans with illness may have widespread WM changes in their brain rather than selective damage. Using low-b DTI setup may result in inconsistent findings due to the insufficient sensitivity to detect microscale changes in the brain. This may also be related to the inconsistencies between our low-b DTI results and the findings of increased axial diffusivity (AD) in IFOF (Rayhan et al., 2013; Chao et al., 2015). Low-b DTI setup can be more useful for investigating neurodegeneration in GW veteran's later life.

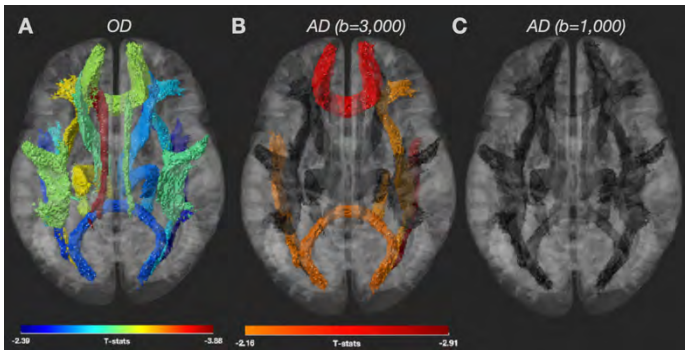


Figure 5. Group statistical results between GWI cases and controls (Kansas definition). Panel A shows group comparison results from OD. Panel B shows results from high-b DTI. Panel C shows results from low-b DTI. Color bar shows t-statistics. Color coding is applied to only statistically significant results (correct $p<0.05$).

b. Other imaging and non-imaging markers for GWI: Differences between GWI cases and controls can be also highlighted from other MRI markers. Based on T1-weighted structural MRI, cortical volume measures revealed significant differences in pre- and post-central area. This pattern was consistent across different subgroups. However, only a few other ROIs were highlighted from this measure. We also tested morphological network measures. Morphological network measures were previously used in other studies for detecting potential synchronized structural changes between cortical regions (Evans, 2013). For example, a previous study showed that individuals with different types of dementia exhibited distinct structural network patterns (Seeley et al., 2009). We found that the morphological network measures are sensitive for describing the GWI pathophysiology. Morphological network connections successfully captured differences between GWI cases on controls (Fig. 6A). Pattern overlap between morphological measures and diffusion MRI markers were found in paralimbic (Fig. 6A, dotted boxes) and ROIs. Since morphological network analyses are based on simple structural scans that are widely available in the neuroimaging research community, this method has great potential for combining different types of large-scale databases within one analysis framework. For better utilization of T1W-MRI scans, we are also suggesting novel T1W-MRI measures as sensitive markers for detecting subtle structural changes of the brain that could not be detected by conventional measures.

We also compared diffusion MRI measures with resting fMRI networks (RSNs) and confirmed strong associations between microstructural deterioration functional networks (Figure 6B). This suggests that tissue microstructural changes in GWI veterans can directly affect functional integrity of their brain. We confirmed consistent findings with Gopinath et al. (2019) that language and dorsal-

attention networks, and sensory-motor networks are disrupted in GWI. Moreover, similar to our diffusion biomarker results (Cheng et al., 2020), we also confirmed distinct alteration patterns in RSNs, which may be specific to different exposure factors (Fig 6C). While dorsal-attention or somato-motor network were consistently shown in most subgroups, salience network (SN) was additionally disrupted in GWI veterans who reported exposure to pesticides, anthrax vaccine or pyridostigmine bromide pill. Also, altered default mode network (DMN) was confirmed in GWI with chemical biological weapon (CBW), chemical alarms, and enemy vehicle exposures. RSNs were also clearly associated with subject symptom scores (Figure 6D). This suggests that alterations in functional network dynamics within and between different regions of the brain can be a key contributor of flexible behavior or illness symptoms in GWI.

Subgroup specific patterns were also found in blood cytokine markers. Among all, TNF alpha receptor I and Proinflammatory cytokine markers were associated with chemical biological weapon exposure, fogged, cream and pesticides Figure 6C. Further, both hd-MRI and RSNs were associated with these cytokine markers in some subgroups (figure 6E), suggesting the presence of exposure-specific pathophysiology or cross-talk between CNS and the innate immune system.

c. Machine learning classification for Kansas and CDC GWI criteria: In our first ML model (i.e., based on RDF), the highest classification performance was 79% accuracy from a combination of WM ND and Cytokine markers. This

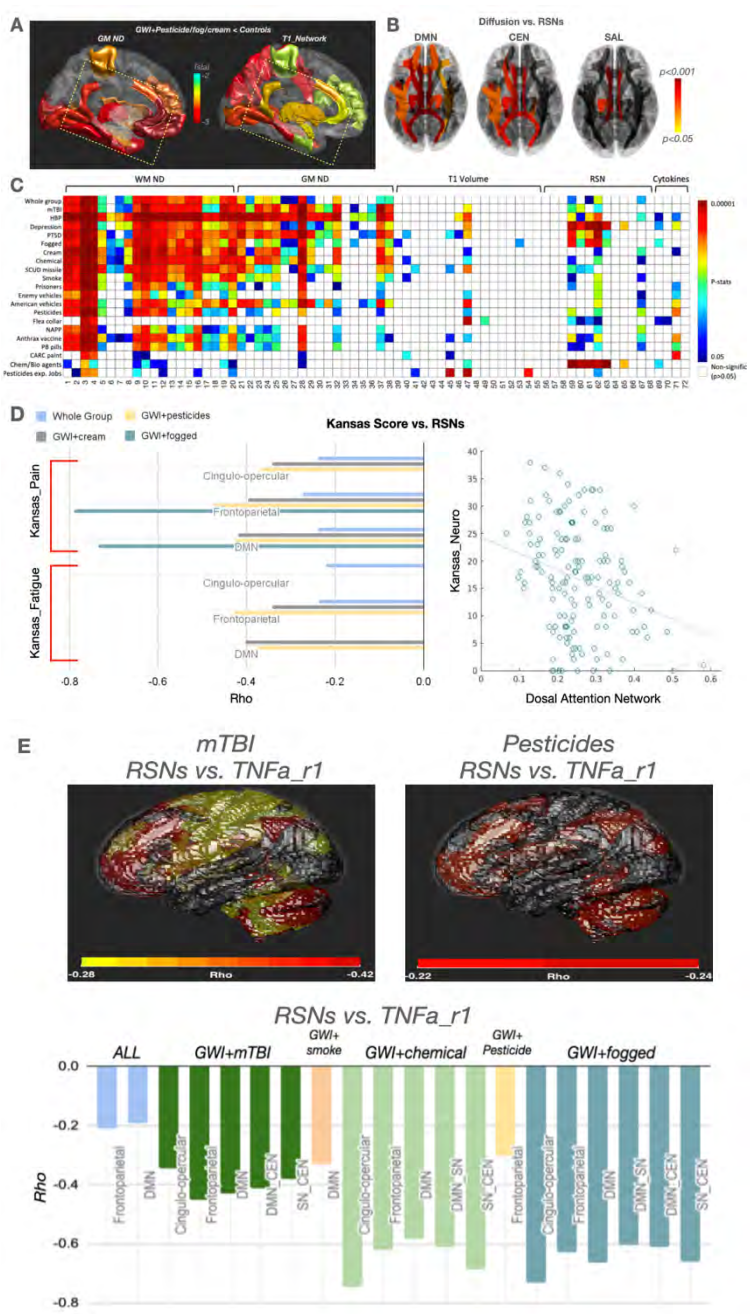


Figure 6. Other MRI imaging and non-imaging features. Panel A shows 3D rendering of GM ND and T1 network features in GWI cases with pesticides/fogged/cream exposure. Limbic/paralimbic regions are marked in yellow dotted box. Panel B shows correlation results between WM ND and RSN features. Left side shows default mode network results, middle shows central executive network results, and the right side shows salience network results. Panel C shows heat map combining hd-MRI, T1 structural, RSN, and blood cytokine features and their subgroup patterns. Rows are different exposures/symptoms and columns are different biomarkers. Statistically significant group differences between subgroups and controls are marked in different colors (please see legends under the heatmap). Distinct subgroup specific feature profiles can be confirmed from this map. Panel D shows correlation between RSNs and Kansas scores. Rho's are plotted in left bar graph. Scatter plot in the right shows correlation between dorsal attention network and Kansas neuro score. Panel E shows 3D rendering of correlation between RSNs and TNF alpha receptor I in mild TBI (left) and Pesticides subgroups (right). Bar graph shows distinct correlation profiles in different subgroups.

model was updated to the CCF model in project year 2. We added GM micro-diffusivity measures and a new machine learning classifier (RDF-CCF). Classification tests were performed based on 91 subjects. Among these dataset, 13 to 15 subjects in each group were randomly subsampled 200 times for training the classifier and rests were used as test dataset for calculating performances. In these almost 2 times larger samples compared to the last year's sample size, the highest classification performance was confirmed in the classifier based on the combined features (ND/OD + DTI + GM volume) in 80% accuracy level. With the same features, GWI classification based on CDC criteria showed 74% accuracy.

Reinforcement learning: In project year 3, we added a reinforcement learning framework. Reinforcement Learning (RL) is one of the ML techniques that enables an agent to learn through an interactive environment (i.e., sharing experiences between agents and also accounting for their previous experiences) by trial and error. Combining with the bee swarm algorithm, our classifier learns to search for a solution (i.e., resulting feature list) that maximizes the reward (i.e., higher classification accuracy) through multiple iterations. This concept is to upgrade a simple local search to a more adaptive and efficient search for the final solution. Our new machine learning framework consists of 3 parts - 1) a prior-based set-up of feature space, 2) key feature selection based on the reinforcement learning, and 3) classifier building based on the selected key feature subsets. In the first part, each imaging feature domain is used for initializing the feature space. The second part of this model is to iteratively search the key feature candidates in the preset feature spaces within the training dataset for potentially better ML outcomes. The last part of this method builds the classification model. From the RL-based feature selection in the training dataset, top candidate feature vectors are built into the KNN, support vector machine, and RDF classifiers. We used a majority voting strategy to merge key solutions to have unbiased classification results (Guan et al., 2020). In ML based on single imaging modality features, WM OD showed the best performance (Figure 7A). NDI features provided better performances than DTI measures. Among the DTI measures, mean diffusivity (MD) was the best feature for ML.

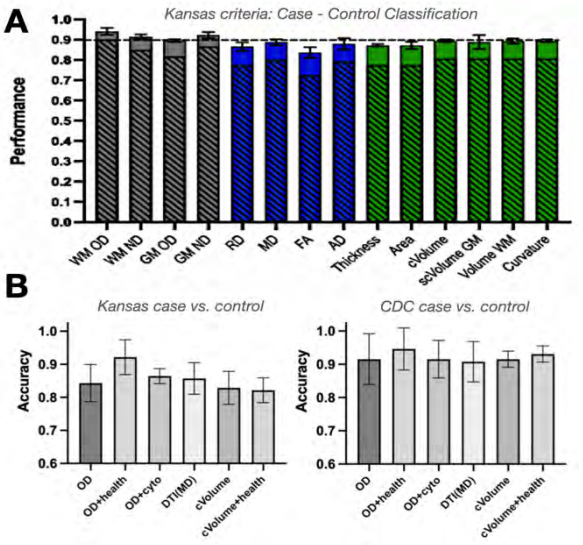


Figure 7. ML classification testing results. Panel A shows RL classification results on Kansas case and controls. The results were based on 119 subject data. Panel B shows CCF+SMOTE results based on 146 subject data. Left side bar graph plot shows results on Kansas criteria. Right side bar graph plot shows classification results on CDC criteria.

CCF with SMOTE (CCF+SMOTE): During the NCE period, we also updated the CCF method. GWIC MRI data is highly imbalanced in GWI case and control groupings (in both Kansas and CDC criteria). This issue can be the cause for poor performance on ML classification problems. This updated CCF performed stratified sampling of training/testing samples and performed initial sampling of features based on different ranges of p-value thresholds (ranging 0.5~0.001). After defining the feature space, SMOTE was added into the framework to perform oversampling on minority training samples. CCF+SMOTE offers fast training speed (~30 minutes per classifier), whereas RL takes 7 to 10 hours in our multi-core computing environment. We used CCF+SMOTE

for the benchmark test between different ML classifiers. In ML based on single imaging modality features for Kansas criterion, CCF+SMOTE from WM OD showed better performance than the best performing model of CCF, which was based on ND/OD, DTI, and GM volume features (80% accuracy). In CDC case vs. control classification, CCF+SMOTE from WM OD above 90 percent accuracy. Overall, classification performances on CDC criterion showed better performances than the results on Kansas criterion (Figure 7B). However, the best classification performance was found in WM OD features combined with subject health profiles (i.e., high blood pressure, depression, and PTSD) in both Kansas (92.1% accuracy) and CDC (94%) classification results. Kansas definition excludes subjects with concomitant medical or psychiatric conditions and also counts more non-neurological symptom clusters (e.g., respiratory or skin) than CDC criteria. These characteristics underlying Kansas criterion can be a source of different ML performance. However, our ML classification results showed that subject health profile information can be a good complement to imaging features for classifying GWI cases in both criteria. Classification performances were more balanced in both criteria by adding the information as a feature. In addition, combining exposure conditions or blood cytokine features to NDI measures showed 1 to 6 percent improvements in classification performance compared to using only NDI measures. CCF with multimodal imaging features did not show significant improvements in our testing. This may be due to too many feature problems in the optimization process. Case subgroups based on health profiles showed 1 to 4 percent improvement, however, exposure-based subgroupings were not beneficial to enhance ML performance. For some exposure conditions, we were not able to test ML due to the small sample size issue. Also, compared to the imaging features, ML based on blood cytokines, cognitive tests, exposures, or health profiles showed 50 to 70 percent performances. These results support our hypothesis that GWI is associated with alterations in the local brain components especially in the microscopic structural integrity and connectivity, and that non-imaging markers may explain residual variations thereby supporting the overall classification performance.

In summary, we developed ML classification frameworks for classifying GWI. Both RL and CCF+SMOTE showed promising results. RL model offers consistent feature selection protocols while CCF+SMOTE offers rapid testing of different parameter setups. We suggest CCF+SMOTE as initial feature space optimization and use RL for enhanced ML modeling. The ML classification framework is embedded in our software package for further research purposes. In collaboration with the Boston Biorepository, Recruitment, and Integrative Network (BBRAIN, PI: Sullivan), PI's group is planning to expand this work to do validations with larger dataset collected in multiple sites. Further, the ML framework has an easily expandable structure to combine new concepts, and it will be a good resource for further GWI research.

d. Software developments: During the NCE period, software packages were updated from the alpha stage version (Figure 8A). All statistical findings, linear modeling results, and ML techniques were embedded into a smart database software package. The package contains following features: 1) 3D visualization of key imaging biomarkers in morphological, connectional, microstructural and resting functional network domains, 2) Highlights of statistical results (Figure 8B), 3) A single-subject level inferencing of brain measures from subject exposure and symptom scores based on multivariate linear modeling (Figure 8C), 4) ML classifier and feature maps for MRI data (Fig. 8D). The package consists of both graphical user interface (GUI) tools and command line processing functions. This software

package aims to support building more practical and robust clinical applications of GWI. PI's team will continuously update functions to support further research works for GWI.

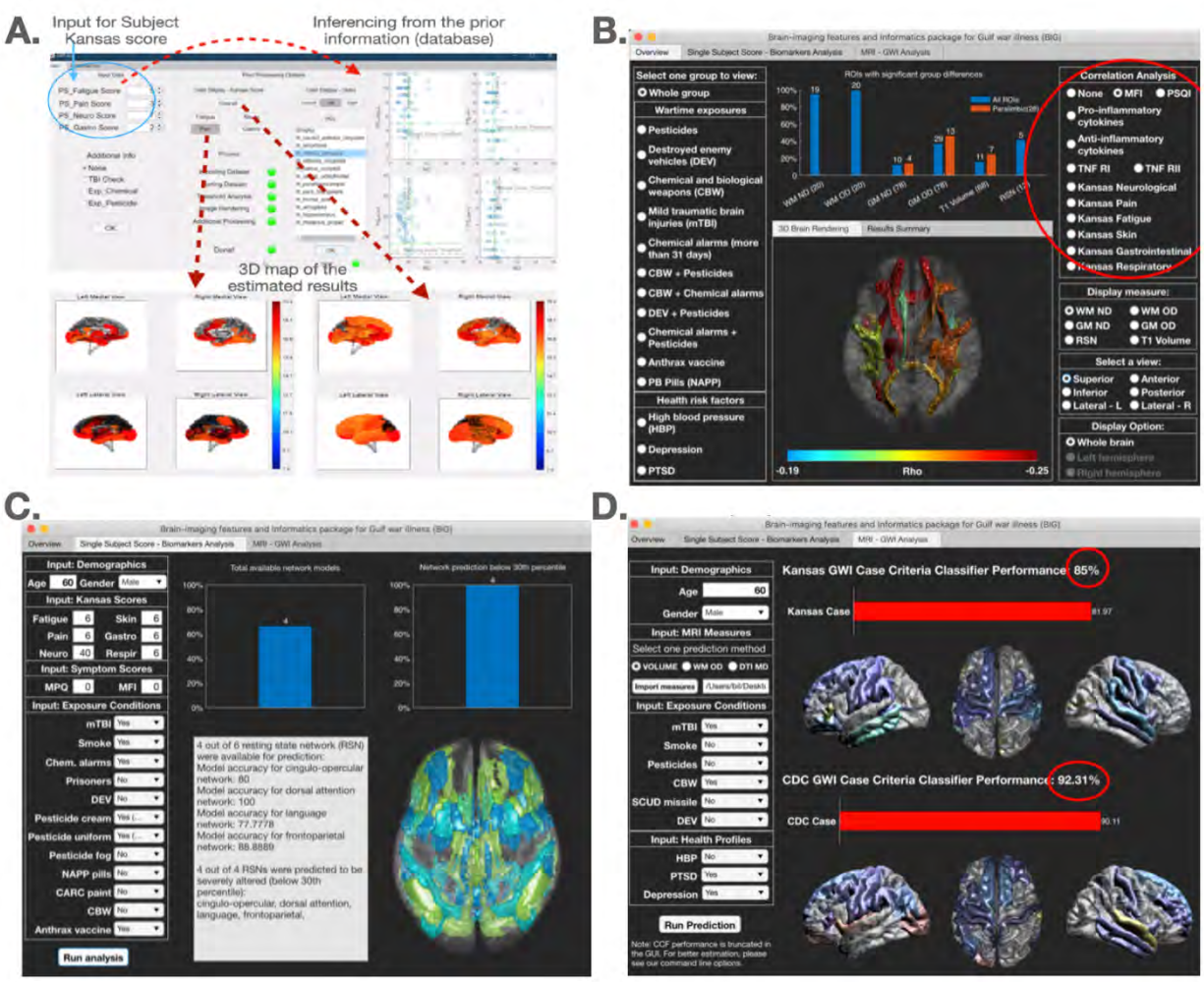


Figure 8. Graphical user interface in the software package. Panel A shows the 1st version of the package developed in project year 3. Panel B-D shows different options offered from updated version. Please see software manual for more information on the updated version (attached in the Appendix section)

What opportunities for training and professional development has the project provided?

Project year 1:

- PI attended an international conference on June 16-22nd 2018. PI shared the preliminary findings to the neuroimaging community. PI also had extensive discussions on diffusion MRI processing with other research groups.

- One-on-one research mentoring was offered to 2 research assistants from the PI. Research assistants learned the basic concept of MRI, brain mapping and statistics processing and had hands-on training using the data from open sources.

Project year 2:

- PI attended an international conference (International Neuropsychological Society) on Feb 21-23rd 2019.

  a. Research Assistant (Ms. Cheng) presented a poster at the meeting.

  b. PI, Dr. Koo, had a meeting with Drs. Linda Chao (UCSF) and Sullivan (BU SPH) at the meeting and discuss GWI neuroimaging results.

  c. Dr. Koo had a meeting with Dr. Nancy Klimas regarding the GWI data.

- PI attended an international conference (World Congress on Brain Injury) on Mar 13-15th 2019.

  a. Dr. Koo was invited for a talk.

  b. Dr. Koo shared neuroimaging findings on GWI veterans with mTBI.

  c. Dr. Koo had a meeting with Drs. Naeser (Boston VA) and Lim (vielight) and discuss potential treatment methods and imaging-based validations.

- PI provided training on computer programming and neuroimage processing to RAs. PI also provided one-to-one mentorship on RAs in the weekly meeting.

-PI attended an in-person meeting of Boston GWIC (PI: Dr. Sullivan) and shared up-to-date findings with other researchers in the consortium. Co-investigators (Drs. Sullivan, Killiany, and Hereen) and consultants in this project (Drs. Steele and Klimas) attended the meeting and discussed the results. Dr. O'Callaghan also attended the meeting and discussed the GWI animal model study.

-PI invited Dr. Marco Loggia for a seminar on September 19th, 2019. Dr. Loggia shared TSPO PET imaging findings in GWI.

Project year 3:

- All in-person laboratory meetings were changed to online zoom meetings. We had two meetings every week for all laboratory members.

- PI's team started weekly remote meetings with Dr. Jaehoon Kim at Samsung medical center, Seoul, Korea. This meeting was to do technical discussions on machine learning and deep learning methods. The meeting ended December, 2020.

-PI planned to attend an international conference (PsychoNeuroImmunology Research Society) on June 3, 2020. However, the meeting was cancelled due to the COVID19.

-PI's team members (research technician, 2 research assistants) have been working on this project. PI provided training on computer programming and neuroimage processing. PI also provided one to-one mentorship on RAs in the weekly meeting.

-PI attended GWIC web meetings (PI: Dr. Sullivan) and shared up-to-date findings with other researchers in the consortium. Co-investigators and consultants in this project (Drs. Steele and Klimas) attended the meeting and discussed the results. Dr. O'Callaghan also participated in the meeting and discussed the GWI animal model study.

- PI present neuroimaging works in VA-DoD Gulf War illness SOTS virtual conference, August 18th, 2018. Co-I, Dr. Sullivan presented at the meeting.

Project year 4 (NCE):

- PI's team maintained weekly online meetings.

- PI invited Dr. David Van Essen, PI of Human Connectome Project, for a lecture on September 24th, 2020. Dr. Van Essen introduced novel approaches on multi-modal neuroimaging and mapping strategies. PI's team members had a one-to-one meeting with Dr. Van Essen and presented their works.

- PI was invited for an on-line lecture at Dept. of Biomedical Engineering, Hanyang university, Seoul Korea on October 26th 2020.

- PI invited Dr. Oscar Liang to give a talk on April 22nd, 2021. Dr. Liang is a collaborator at BBRAIN.

How were the results disseminated to communities of interest?

All the work done in year 1 was reported as part of GWIC External Advisory Board (EAB) meeting to the CDMRP scientific officer and Program manager. Also, cortical morphometry and DTI measurements have been updated and shared to GWIC researchers.

PI introduced multimodal brain mapping concepts for studying brain-immune interactions at a Boston Puerto-Rican Health Study (BPRHS) meeting and helped design imaging protocols for the study.

All the works done in year 2 were reported as part of GWIC annual meeting. Also, all the imaging measures have been shared to GWIC researchers.

Most of the work done in year 3 was reported at VA/DoD virtual meeting (Aug. 18th, 2020). Also, all the imaging measures have been shared to GWIC researchers, DoD officers and veterans who attended the meeting.

In the NCE period, PI introduced high-dimensional diffusion MRI mapping technique and results from GWIC data to students and faculties with biomedical engineering backgrounds (Hanyang University).

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Nothing to report.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Recent studies on GWI suggest that there is a strong brain-immune component to the disorder and that biological measures taken from brain imaging and immuno-genetic measures reflect different but potentially connected aspects of the illness. These measures have mainly only been studied in isolation and the predictive risk of the illness at the individual veteran level have not been high enough to be useful. The goal of this study was to take various promising biological markers of GWI in collaboration with a large ongoing GWI Consortium (GWIC) study (GW120037) who's overall hypothesis is that GWI is associated with altered brain-immune interactions and cross-talk pathways. There were two key concepts applied to this project. One was to bring cutting edge neuroimage processing techniques to extract quality imaging measures with better sensitivity to study brain-immune interaction from GWIC imaging data. The other was to utilize a machine learning framework to incorporate different biomarkers (blood tests, cerebrospinal fluid, brain imaging) for further investigation of the complex interactions that represent GWI etiology. These two different concepts were complementary to each other and incorporated into the project design. From this idea, we could draw extended figure on GWI as follows:

- Multidimensional diffusion MRI mapping revealed that veterans with GWI have clear group specific microstructural profiles in WM connections and limbic/paralimbic regions compared to GW veteran controls. Damage in WM in GWI veterans was associated with micro-scale diffusion components and revealed more widespread patterns compared with previous findings. For studying brain-immune interaction, high-b or complex diffusion MRI may provide more sensitive measures than low-b DTI. Referring back to our animal imaging works, micro-diffusion imaging revealed that it is feasible to discriminate different stages of neuroinflammation in different parts of the brain (Koo et al., 2018). Considering the same measures applied in the veteran's data, results support that microscopic diffusivity fingerprints chronic inflammation in GWI. In addition, our findings on TSPO-PET and diffusion MRI suggest that the high-b value encoded diffusion MRI can be a good alternative imaging method to PET scans. Microscale diffusivity successfully captured changes in brain tissue environment associated with glial activations and provided better explanation of illness symptoms.

- Microstructural profiles in GM can be useful markers for studying the relationships between symptoms and imaging markers.

- Subgroup analysis results support an idea that 'in theater' exposure conditions are an important neurological risk factor to describe and understand the brain health of GW veterans. We confirmed that association between brain imaging and peripheral immune markers resides in specific conditions. This also suggests that significant variations within GWI cases can be explained and decoded into several clusters.

- Combining different types of biomarker data into a cohesive computational model allowed for validating biomarkers that represent GWI and most importantly to map these markers in individual subject space rather than only looking at information at the group level. The impact of identifying

validated and replicated biomarkers of GWI and identifying how predictive each marker is of the other can be invaluable in moving the field forward and for reliably using those biomarkers to predict future treatment trial efficacy at the group and individual veteran level.

- Our ML results also suggest that microscale diffusion measures are key features for classifying GWI. However, results also indicate that the features highlighted from univariate statistical analysis are not always the best solutions for classification. For example, volumetric features did not show significant group differences between GWI cases and controls, however, classification results based on those features were comparable to the results from some diffusion features.

- CDC definition on GWI may have more overlapped information with brain imaging features than Kansas definition. This is because the Kansas definition excludes subjects with concomitant medical or psychiatric conditions and also counts more non-neurological symptom clusters (e.g., respiratory or skin) than CDC criteria. However, Kansas scores are invaluable for studying the relationships between specific symptom clusters and CNS measures. In addition, combining subject health conditions to imaging markers helps enhance classification performance. This also suggests that subject health conditions can be a good complementary information to imaging biomarkers.

- Our results confirmed that there are noticeable intra-group variations within the GWI veteran group. Further discussions on modifying Kansas criteria might help improve its diagnostic value. We suggest using neuroimaging measures as a reference information for the fine-tuning of the criteria.

What was the impact on other disciplines?

Results indicate that illness symptoms in GW veterans mediates the chronic neuroinflammation which can be fingerprinted by microstructural imaging in limbic/paralimbic structures. Alterations in those brain regions has been also highlighted from other chronic inflammatory disorders and thereby can likely be a critical information on understanding the role of neuroinflammation in other diseases such as depression (Richards et al., 2018), fibromyalgia (Albrecht et al., 2018), and so on. From this work, we are suggesting a framework for extracting objective measures and ways to combine different measures to study pathophysiology of the illness. This might also be an effective method to study other diseases.

- It has been demonstrated that there is a correlation between GW illness symptom severity and the occurrence of mTBI among veterans suffering from multiple illness symptoms (Yee et al., 2017). Results support that GW veterans with both mTBI and other GW-relevant exposures have a greater impact on the microstructural integrity in the brain compared to subjects without mTBI. This suggests that potentially brain damaging exposure could be present in veterans exposed to both mTBI and other risk factors.

- Microstructural profiles in the brain can also provide key information to understanding neuronal degenerative and/or regenerative mechanisms in normal and pathological aging processes. Synaptic loss and microgliosis have been demonstrated to be the earliest features, even preceding neuronal loss and tangle formation, in a tauopathy mouse model, suggesting the importance of understanding the microstructural profiles that characterize the early events of human tauopathies (Yoshiyama et al., 2007). Furthermore, alterations in synaptic/dendritic compartments are tied to tau pathology in both Alzheimer's disease (AD) and frontotemporal dementia (FTD), while linkage to Aß was found only in AD (Ittner et al., 2018).

- In this project, we have found promising "hints" that the novel multi-compartmental diffusion markers can provide key information to understand the neuro-immune interactions by capturing microstructural change. Considering the neuroimmune component in Alzheimer's disease and related dementia, the data obtained here suggest that these markers can be valuable for deriving microstructural information in those cohorts to obtain further insights in-vivo.

-Predicting the progression of AD is challenging, and the classifiers generally achieved an accuracy ranging from 70-80% using only conventional MRI features. The ML framework using novel MRI markers suggested in this project can be a potential solution to improve the performance.

What was the impact on technology transfer?

The software package is opened to the GWI research community through collaborations to support building more practical and robust clinical applications of GWI.

What was the impact on society beyond science and technology?

- Providing the objective markers with a sophisticated computational framework on a single subject level diagnosis can help improve clinical decisions and further development of clinical protocols for finding better cure for GWI. Biomarkers can also be used to validate a patient's self-report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

We initially planned for analyzing CSF biomarkers, however, the collected sample size was not sufficient for the analysis. To address this issue, we used TSPO-PET as an alternate CNS immune marker and applied it to our validation work on diffusion markers. Selective diffusion markers were highly correlated with TSPO-PET and applied for ML classification.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

1. **Koo BB***, Michalovicz LT, Calderazzo S, Kelly KA, ***Sullivan K***, Killiany RJ, O"Callaghan JP. Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. Brain Behav Immun. 2017 Aug 04. (*1st and corresponding author)

2. Cheng CH, **Koo BB***, Calderazzo S, Quinn E, Aenlle K, ***Steele L***, Klimas N, ***Krengel M***, Janulewicz P, Toomey R, Michalovicz LT, Kelly KA, Heeren T, Little D, O'Callaghan JP, ***Sullivan K***. Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. Brain Behav Immun. 2020 Oct; 89:281-290. *corresponding

3. Guan Y, Cheng C-H, Chen W, Zhang Y, Koo S, ***Krengel M***, Janulewicz P, Toomey R, Yang E, Bhadelia R, ***Steele L***, Kim J-H, ***Sullivan K***, **Koo BB***. Neuroimaging Markers for Studying Gulf-War Illness: Single-Subject Level Analytical Method Based on Machine Learning. Brain Sciences. 2020; 10(11):884. doi:10.3390/brainsci10110884 PMCID: PMC7699718 *corresponding

4. Keating, D., Zundel, C.G., Abreu, M., ***Krengel, M***., Aenlle K., Nichols, D., Toomey, R., Chao, L.L., Golier, J., Abdullah, L., Quinn, E., Heeren, T., Groh, J., **Koo, B.B.,** Killiany, R., Loggia L.M., Younger, J, ***Baraniuk, J.***, Janulewicz, P, Ajama, J. Quay, M., Baas, P., Qiang, L., Conboy, L., Kokkotou, E., O'Callaghan, J., Steele, L., Klimas, N., ***Sullivan, K****, Boston Biorepository, Recruitment and Integrative Network (BBRAIN): A Resource for the Gulf War Illness Scientific Community, Life Sciences, Accepted.

5. ***Steele L****, Klimas N, ***Krengel M***, Quinn E, Toomey R, Little D, Abreu M, Aenlle K, **Koo BB**, Killiany R, Janulewicz P, Heeren T, Clark AN, Ajama J, Cirillo J, Buentello G, Lerma V, ***Sullivan K***, Brain Sciences, Accepted.

6. Cheng C-H, Alshelh Z, Guan Y, Loggia LM, ***Sullivan K***, **Koo BB*,** Association of the tissue microstructural diffusivity and translocator protein PET in Gulf War Illness, Brain Behav Immun. Health. In-review (2nd round) - draft attached in the end of this document. *corresponding

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

1. **Koo** et al., A comparison of different brain connectivity markers for classifying Gulf-war illness, International Society of Magnetic Resonance in Medicine (ISMRM), Abstract, Poster Presentation, 2018

2. Multimodal MRI imaging protocol introduced to Philips Medical Systems in ISMRM user group meeting (slide attached).

3. Cheng J, Little D, **Steele L**, Heeren T, Killiany R, **Sullivan K**, **Koo B***, Preliminary evaluation of diffusion imaging features for classifying veterans with Gulf war illness, International Neurological Society, Abstract, Poster Presentation 2019

4. **Koo BB**, Presentation on Gulf War Illness Imaging Protocol in the External Advisory Board (EAB) meeting, June 14th 2018.

5. **Koo B**, Cheng C, Little **D, Steele** L, Heeren T, **Sullivan K**, Mild TBI during war is associated with further microstructural alterations in the cortical gray and white matter in 1991 Gulf War Veterans with Gulf War Illness, World Congress on Brain Injury, Oral Presentation 2019.

6. Clara G. Zundel, R. Killiany, **B. Koo**, **M. Krengel**, R. Toomey, J. Ajama, P. Janulewicz-Lloyd, M. Abreu, T. Heeren, E. Sisson, D. Little, **L. Steele**, N. Klimas and K. Sullivan, Objective Biomarkers of Gulf War Illness: White Matter Microstructural Integrity, Cognition, and Blood Biomarkers in Gulf War Veterans, International Neurological Society, Abstract, Poster Presentation 2019

7. **Koo BB**, Multi-modal diffusion MRI and Machine Learning, Invited Seminar, Dept. of Biomedical Engineering, Hanyang University, Seoul Korea. 2020.

8. **Koo BB**, VA-DoD Gulf War Illness SOTS Virtual Conference 2020 Oral Presentation - Multi-modal MRI imaging Boston GWIC Consortium

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Multi-modal MRI processing pipeline was designed for studying GWI and is available for BBRAIN data.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Brain-imaging features and Informatics package for Gulf war illness (BIG): available through collaboration.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Bang-Bon Koo*
Project Role:	*Principal Investigator / No Change*

Name:	*Kimberly Sullivan*
Project Role:	*Co-investigator / No Change*

Name:	*Ron Killiany*
Project Role:	*Co-investigator / No Change*

Name:	*Timothy Heeren*
Project Role:	*Co-investigator / No Change*

Name:	*Jasmine Cheng*
Project Role:	*Research Technician*

Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Ms. Cheng has been working on software programming, data processing and organizing the imaging measurement outcomes.*
Funding Support:	

Name:	*Guan Yi*
Project Role:	*Research Assistant (Graduate Student)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Ms. Yi performed work on working on data processing and programming.*
Funding Support:	*Started supporting on July 2019.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

- Organization Name: Baylor College of Medicine
Location of Organization: *One Baylor Plaza, Houston TX 77030*

Partner's contribution to the project: Consultant
Financial support: none
In-kind support *(e.g., partner makes software, computers, equipment, etc., available to project staff)*: Discussions on the project
Facilities: None
Collaboration: responsible for managing the Texas site data and consulting on GWI symptoms on this project.
Personnel exchanges
Other.

-Organization Name: Nova Southeastern University
Location of Organization: 3301 College Ave, Fort Lauderdale, FL 33314
Partner's contribution to the project: Consultant
Financial support: none
In-kind support *(e.g., partner makes software, computers, equipment, etc., available to project staff)*: Discussions on the project
Facilities: None
Collaboration: consulting immunogenetics part on this project.
Personnel exchanges
Other.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to report.

9. APPENDICES:

a. References cited

b. Software manual

c. Publication & Presentation materials

References

Albrecht D. S., Forsberg A., Sandström A., Bergan C., Kadetoff D., Protsenko E., et al. (2019). Brain glial activation in fibromyalgia–A multi-site positron emission tomography investigation.BrainBehav.Immun. 75 72–83. 10.1016/j.bbi.2018.09.018

Alshelh, Z., Albrecht, D. S., Bergan, C., Akeju, O., Clauw, D. J., Conboy, L., ... & Loggia, M. L. (2020). In-vivo imaging of neuroinflammation in veterans with Gulf War illness. *Brain, behavior, and immunity*, *87*, 498-507.

Breiman. Random forests. Machine Learning , 45(1):5–32, 2001

Chao, L. L., 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.

Cheng, C. H., Koo, B. B., Calderazzo, S., Quinn, E., Aenlle, K., Steele, L., ... & Sullivan, K. (2020). Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. *Brain, behavior, and immunity*, *89*, 281-290.

Evans, A. C. (2013). Networks of anatomical covariance. *Neuroimage*, *80*, 489-504.

Fischl, B. and Dale, A., Measuring the Thickness of the Human Cerebral Cortex from Magnetic Resonance Images. Proceedings of the National Academy of Sciences, 97:11044-11049. 2000.

Guan, Y., Cheng, C. H., Chen, W., Zhang, Y., Koo, S., Krengel, M., ... & Koo, B. B. (2020). Neuroimaging Markers for Studying Gulf-War Illness: Single-Subject Level Analytical Method Based on Machine Learning. *Brain sciences*, *10*(11), 884.

Ittner A, Ittner LM: Dendritic Tau in Alzheimer's Disease. Neuron 99:13-27, 2018

Koo, B. B., Michalovicz, L. T., Calderazzo, S., Kelly, K. A., Sullivan, K., Killiany, R. J., & O'Callaghan, J. P. (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.

Pasternak, O., Sochen, N., Gur, Y., Intrator, N. & Assaf, Y. Free water elimination and mapping from diffusion MRI. *Magn. Reson. Med.* **62**(3), 717–730 (2009).

Rainforth, T., & Wood, F. (2015). Canonical correlation forests. *arXiv preprint arXiv:1507.05444*.

Rayhan, R. U., Stevens, B. W., Timbol, C. R., Adewuyi, O., Walitt, B., VanMeter, J. W., & Baraniuk, J. N. (2013). Increased brain white matter axial diffusivity associated with fatigue, pain and hyperalgesia in Gulf War illness. *PloS one*, *8*(3), e58493.

Richards E.M., Zanotti-Fregonara P., Fujita M., Newman L., Farmer C., Ballard E.D., Machado-Vieira R., Yuan P., Niciu M.J., Lyoo C.H., et al. PET radioligand binding to translocator protein (TSPO) is increased in unmedicated depressed subjects. EJNMMI Res. 2018;8:57.

Seeley, W. W., Crawford, R. K., Zhou, J., Miller, B. L., & Greicius, M. D. (2009). Neurodegenerative diseases target large-scale human brain networks. *Neuron*, *62*(1), 42-52.

Smit S.M. h, Jenkinson M., Johansen-Berg H., Rueckert D., Nichols T.E., Mackay C.E., Watkins K.E., Ciccarelli O., M.Z. Cader, Matthews P.M., and Behrens T.E.J.. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. NeuroImage, 31:1487-1505, 2006.

Yee MK, Seichepine DR, Janulewicz PA, Sullivan KA, Proctor SP, and 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 Rehabil. Sep-Oct;31(5):320-8.

Yendiki A, Panneck P, Srinivasan P, Stevens A, Zöllei L, Augustinack J, Wang R, Salat D, Ehrlich S, Behrens T, Jbabdi S, Gollub R and Fischl B (2011) Automated probabilistic reconstruction of white-matter pathways in health and disease using an atlas of the underlying anatomy. *Front. Neuroinform.* 5:23. doi: 10.3389/fninf.2011.00023

Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, et al: Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron 53:337-351, 2007

Zhuo, J., Xu, S., Proctor, J. L., Mullins, R. J., Simon, J. Z., Fiskum, G., & Gullapalli, R. P. (2012). Diffusion kurtosis as an in vivo imaging marker for reactive astrogliosis in traumatic brain injury. NeuroImage, 59(1), 467–477. http://doi.org/10.1016/j.neuroimage.2011.07.050

Brain-imaging features and Informatics package for Gulf war illness (BIG)

August, 2021

Table of Contents

Overview

BIG is a cross-platform, interactive graphical user interface (GUI) designed for the Gulf War Illness (GWI) research community to provide GWI-specific clinical inferences based on robust clinical and neuroimaging data from multiple GWI research sites. The BIG GUI is designed for the display, analysis, and diagnostic inferencing of structural, diffusion, and functional measures for GWI, using subject-specific GW-related and health-related information.

In the current version, BIG provides a summary of measures from all subjects used for developing the GUI and has additional options for users to input new subject data, either clinical symptom scores or specific neuroimaging data, to provide inferences on GWI-related brain alterations or predictions on GWI case status.

Installing the GUI:

Download and unzip MyAppInstaller_web.zip from GitHub and follow the installation instructions.

Requirements:

MATLAB Runtime Compiler R2021a (9.10) (Note: This will be downloaded if you follow the instructions in the MyAppInstaller_web.zip)

To start the GUI:

Double click the GUI icon once installation is complete.

Note: The BIG GUI is still being developed and we are working on improving its functionality and practicality for the GWI research community. Currently we are sharing the OSX compiled version, Windows and Linux versions will be available in the next distribution. To report any errors or provide any feedback, please email Jasmine Cheng at chiahsin@bu.edu.

Gulf War Illness Consortium results overview - Step 1: Select one group for display

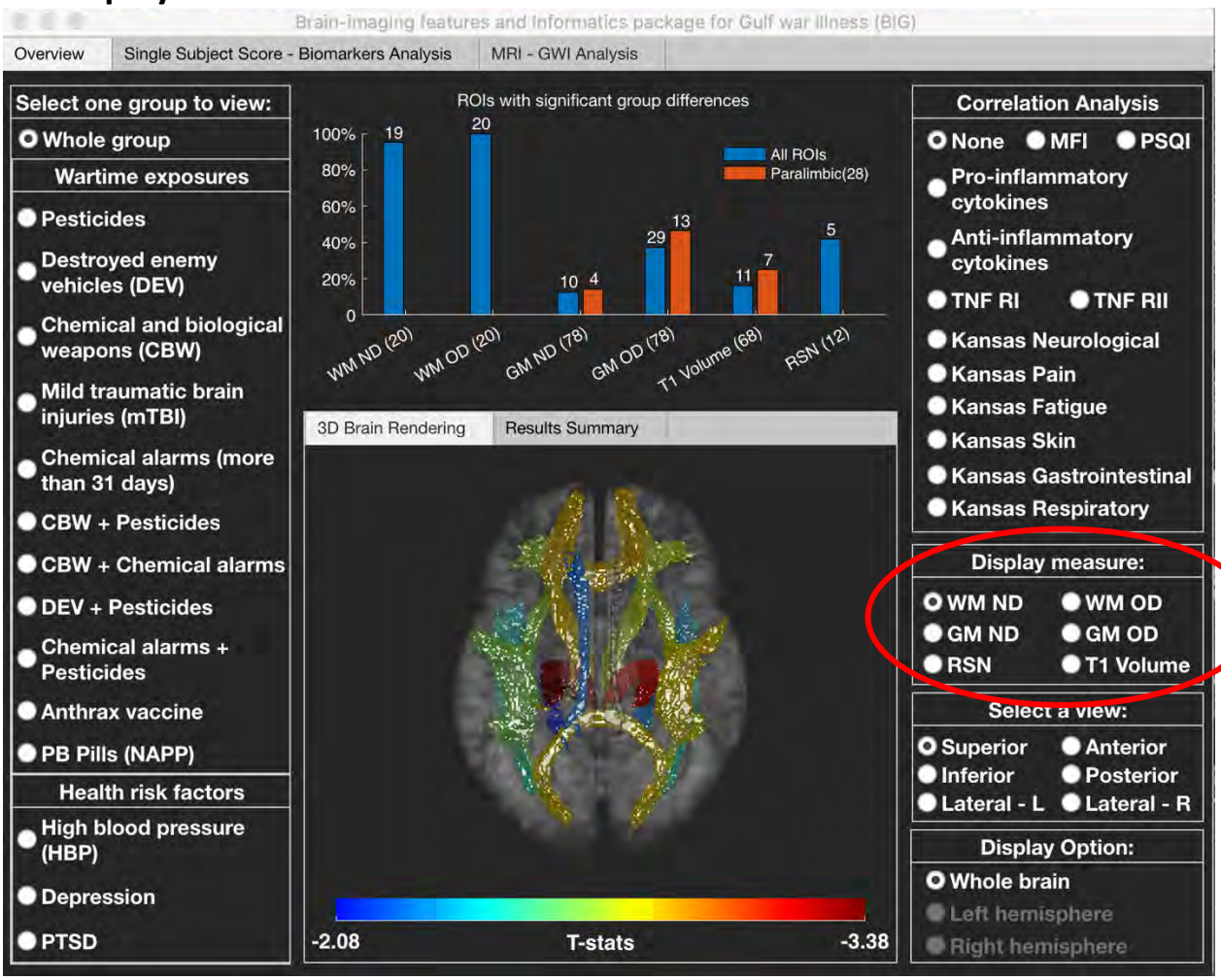
The Overview tab lists 15 groups for display, including a whole group analysis that compared GWI cases and controls based on the Kansas GWI criteria. The other 14 subgroups compared GWI cases (KS criteria) with specific wartime exposures or health risk factors to GW controls. 11 GW-related wartime exposures were defined by self-reported questionnaire responses that asks GW veterans about their experiences/exposures to specific wartime exposures (Steele, 2000). 3 health related risk factors were defined by GW veterans' self-reported responses on whether they are diagnosed with the specific health risk factor. Some wartime exposures were combined to provide a more holistic result.

All group comparisons are performed by age and sex-controlled general linear models, diffusion measures (ND&OD) are controlled for site variances additionally.

Once users select a group to view, the bar graph in the middle panel displays the percentage of regions of interest (ROI) that showed significant patterns in group analysis ($p<0.05$), the number on top of each bar lists the actual number of ROIs that showed significant group differences. The numbers in parentheses indicate the total number of ROIs for each measure. For gray matter (GM) and T1 measures, it also displays an additional red bar for 18 paralimbic ROIs

(caudal anterior cingulate, entorhinal, fusiform, isthmus cingulate, parahippocampal, posterior cingulate, precuneus, rostral anterior cingulate, insula).
The default selection shows the Whole group bar graph, and the WM ND superior whole brain results in the bottom panel.
When None is selected for the Correlation Analysis panel, the color bar displays the t-statistic of the significant ($p<0.05$) ROIs.
Lastly, ROIs that showed significant group differences after doing the false discovery rate (FDR) test are given a higher opacity value in the brain rendering figure (Benjamini and Hochberg, 1995).

Gulf War Illness Consortium results overview - Step 2: Select one measure for display



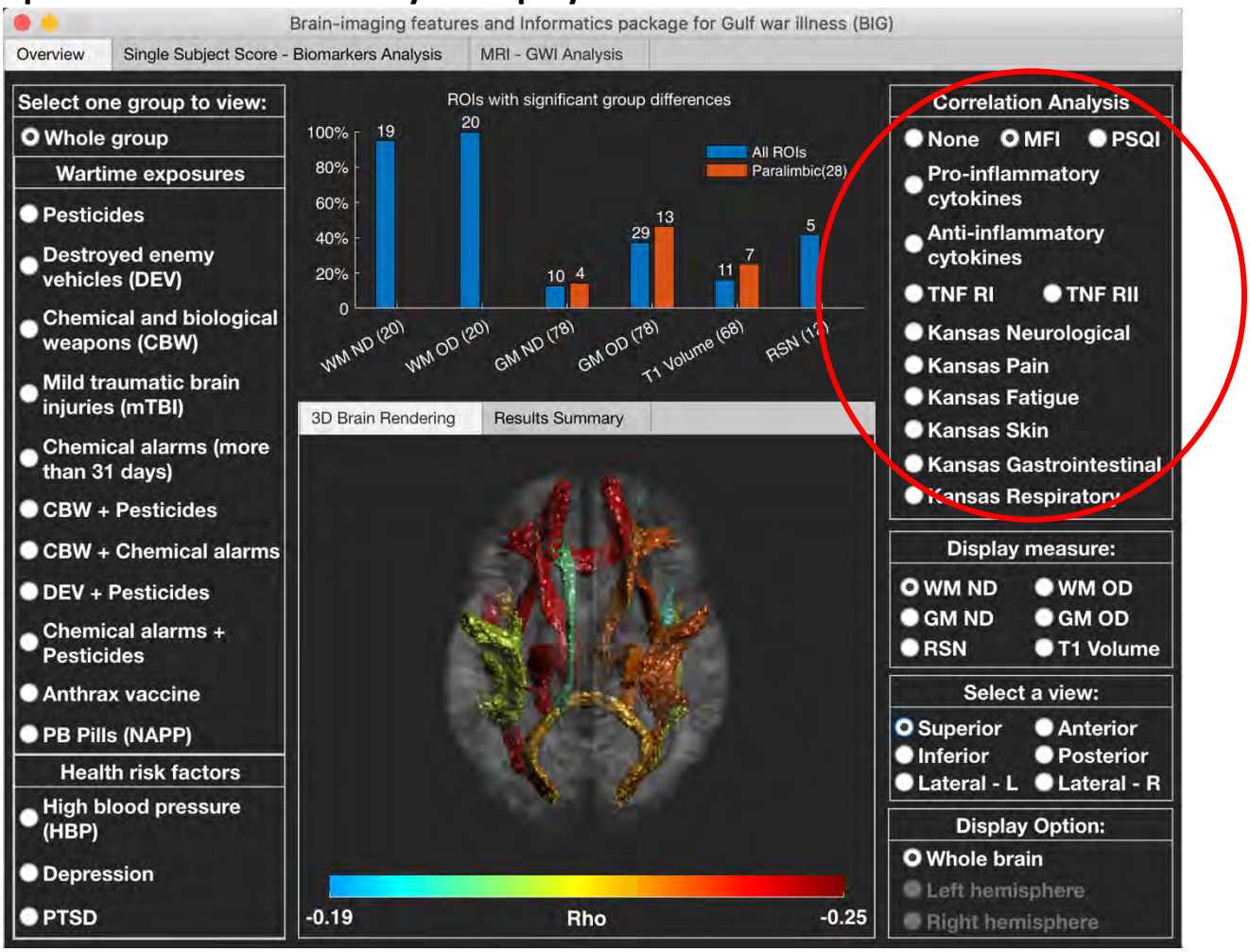
6 different brain imaging measures are available for users to select for display: GM and WM neurite density measures (ND), orientation dispersion measures (OD), T1 volumetric measures, and resting state functional network measures (RSN).
20 WM tracts are defined based on the JHU WM tractography atlas.
68 T1 ROIs are defined based on DKT cortical parcellation atlas.
78 GM ROIs are defined by the 68 cortical ROIs from the DKT atlas plus 10 subcortical ROIs (5 per hemisphere: hippocampus, thalamus, amygdala, anterior amygdala, putamen).
12 RSNs are average resting state functional network connectivity measures defined by the Cole-Anticevic Brain-wide Network Partition (CAB-NP; Ji et al., 2019).
ND and OD diffusion imaging measures that estimate microscale intracellular diffusivity and the spatial configuration of neurites, respectively (Cheng et al., 2020).
T1 volume measures are processed and extracted from FreeSurfer T1 reconstruction pipeline (Fischl, 2012).

Gulf War Illness Consortium results overview - Step 3: Select brain view

For all six measures, users have 6 view options: superior, inferior, anterior, posterior, left lateral (Lateral -L), and the right lateral (Lateral - R) view.

For GM and T1 measures, users can also opt to view only the left or right hemisphere for better visualization of subcortical and medial brain structures.

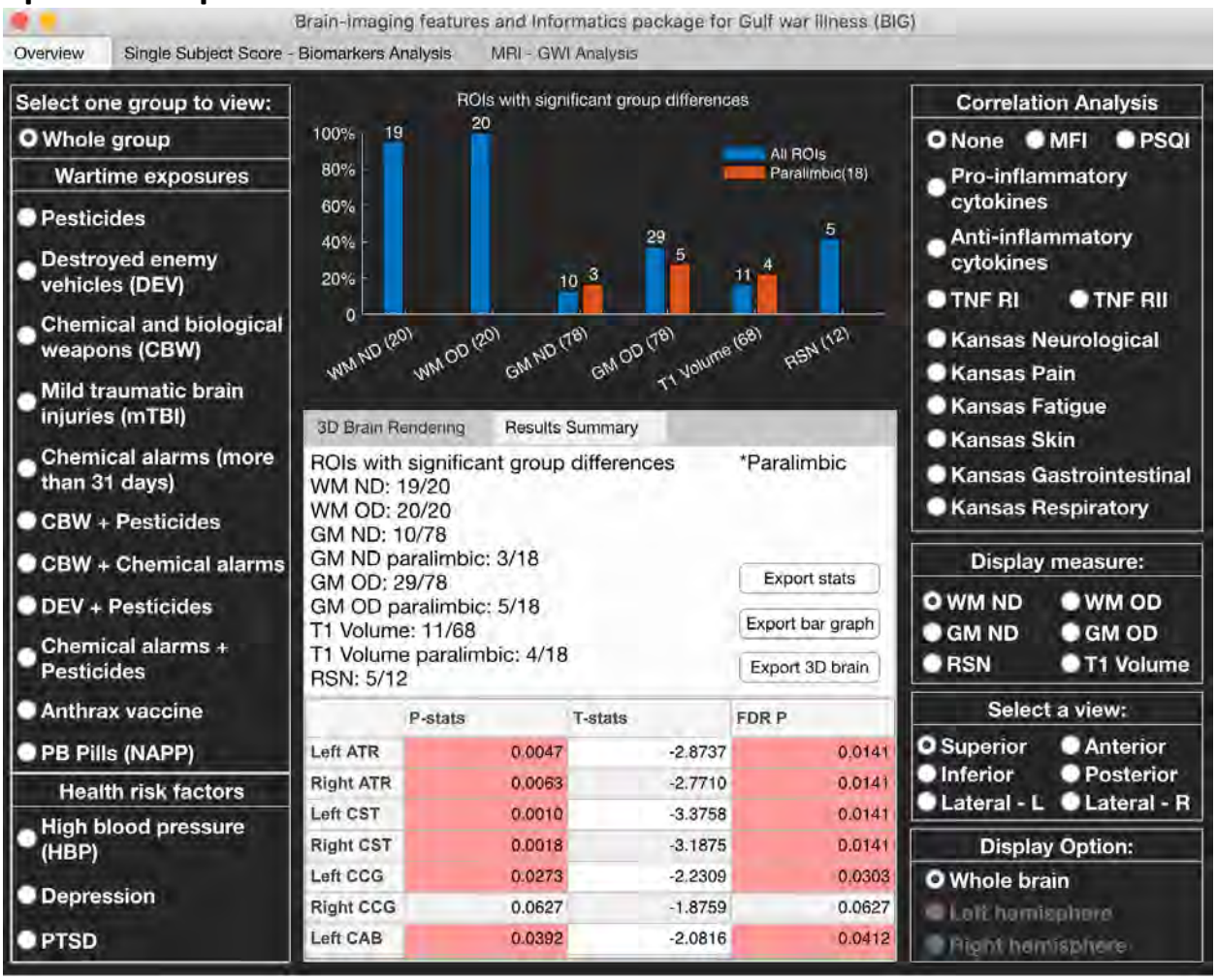
Gulf War Illness Consortium results overview - Step 4: Additional result options – correlation analysis display



In addition to group comparisons, users can view the correlation results between the selected brain imaging measure and 1 out 9 different GW-related symptomatic measures: Multidimensional Fatigue Inventory (MFI), Pittsburgh Sleep Quality Index (PSQI), pro- and anti-inflammatory, TNFR1, and TNFR2 cytokines, Kansas GWI criteria neurological, pain, fatigue, skin, gastrointestinal, and respiratory domains.

Once users select a measure in the Correlation Analysis panel, the brain rendering figure in the middle will display the ROIs that are significant in both group analysis and correlation analysis, and the color bar will display the range of Pearson's rho values of the significant ROIs.

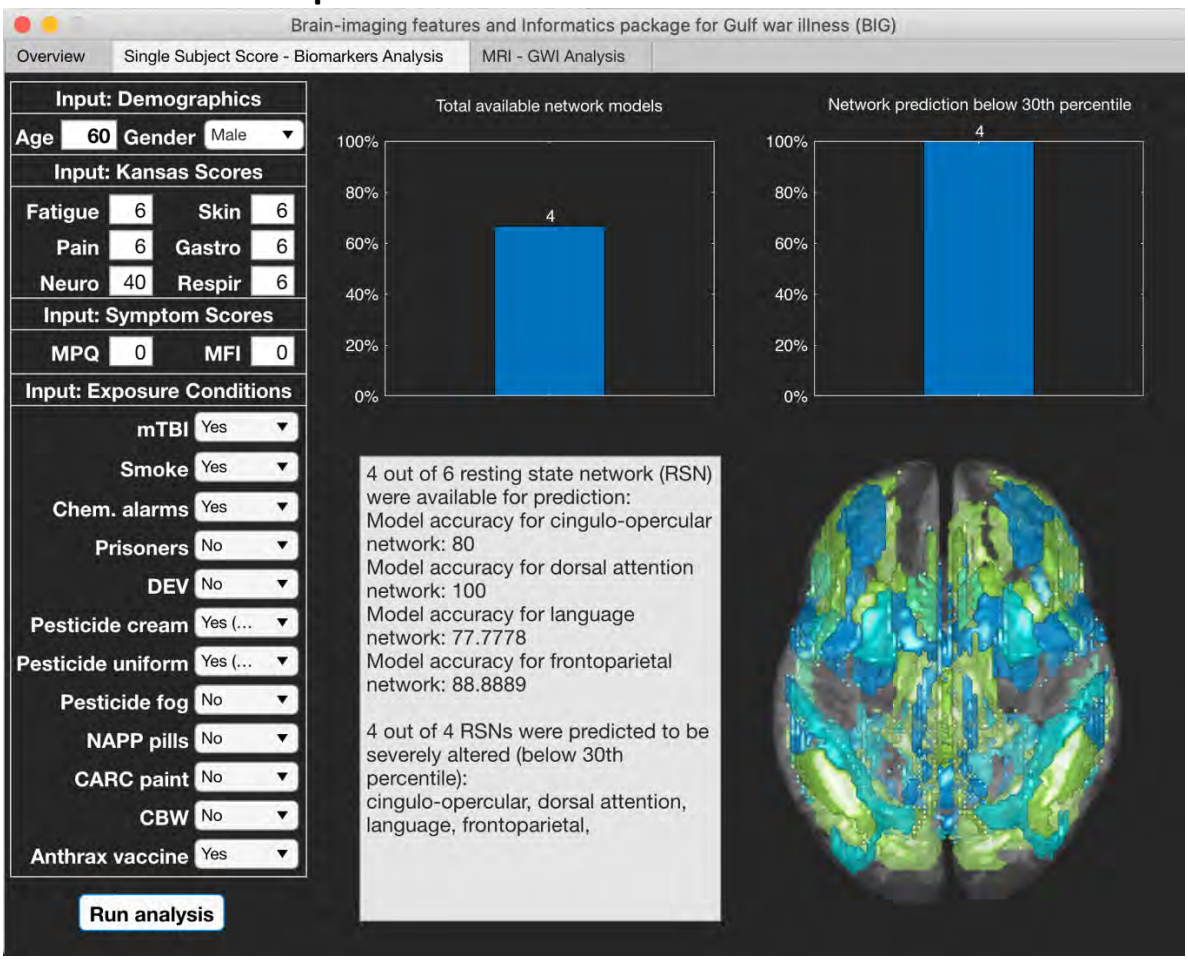
Gulf War Illness Consortium results overview - Step 5: Additional result options – export results



The Results Summary tab lists out the bar graph results for the selected group in text format and displays the statistical results of the selected measure as a scrollable table. Significant ($p<0.05$) ROIs are highlighted in red and paralimbic ROIs are marked with an asterisk (*) sign next to the ROI names.

Lastly, users have the option to view the results in text/table summarized format in the "Results Summary" tab, export statistical results as a csv file, or export the bar graph or 3D brain figures as png files.

Single subject resting state network inferencing – Subject demographics and clinical data input



The Single Subject Score – Biomarker Analysis tab allows users to simulate brain alterations based on single subject demographics, symptom scores, and available wartime exposure information.

In the current version, we provide simulation based on generalized linear models for RSN measures. The models were built using subjects from GWIC, controlled for age and gender. Networks of interest included the somatomotor, language, frontoparietal, cingulo-opercular, and default mode networks.

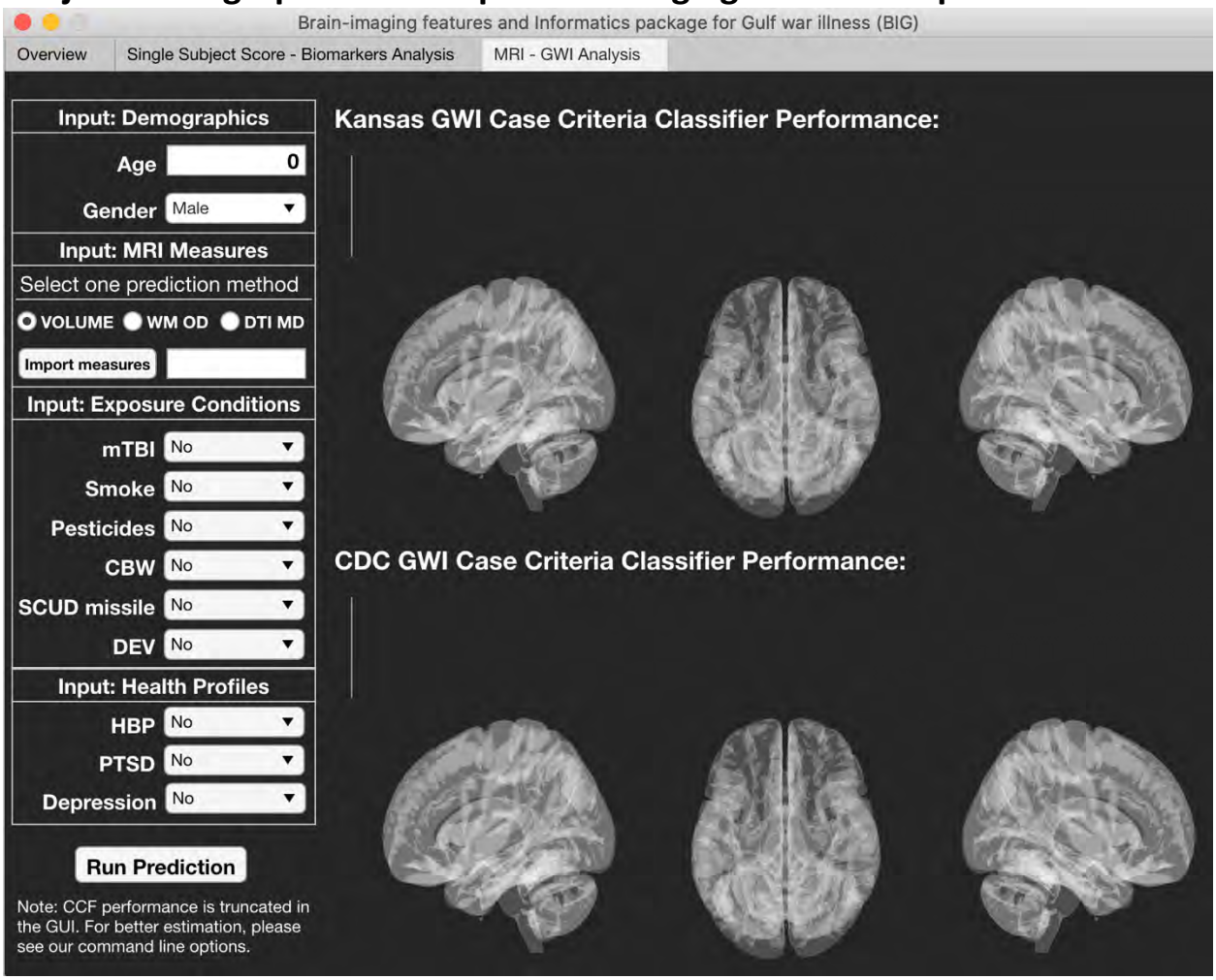
Available models were prescreened based on correlation analyses between RSNs and each self-reported symptom score, using each wartime exposure as subgroup criteria. Correlations with p-values less than 0.001 and rho values greater than 0.3 or less than -0.3 were selected and built as available models.

The GUI will search through available models based on user's inputs and select the best model for each RSN (lowest p-value) based on significance levels from correlation analyses and display the total available models along with prediction results, listing each available RSN model and each model's accuracy level.

For "Pesticide cream" and "Pesticide uniform" exposures, in addition to the binary (Yes and No) options, users have an extra option if the subject's exposure duration is longer than 31 days. If

this option is selected, the BIG GUI will search through all available models for both “Yes (any duration)” and “Yes (31 days or more)” and return the best model prediction.
In addition, we used the 30^{th} percentile as a threshold: network measure lower than the threshold is considered to be severely altered. The GUI displays the number of RSN below the threshold and list each RSN in the textbox.
The GUI provides a brain rendering of the RSNs below the threshold, each network is plotted in a different color.
Once users fill in all available information, click the “Run analysis” button and the GUI will display the results on the side.

Machine learning classification for Gulf War Illness case prediction - Step 1: Subject demographics data input and imaging measure import



The MRI – GWI Analysis tab is designed for users who have diffusion or T1 volumetric data to import their single subject measure, select applicable wartime exposure or health risk factors to predict the GWI status of the given subject.

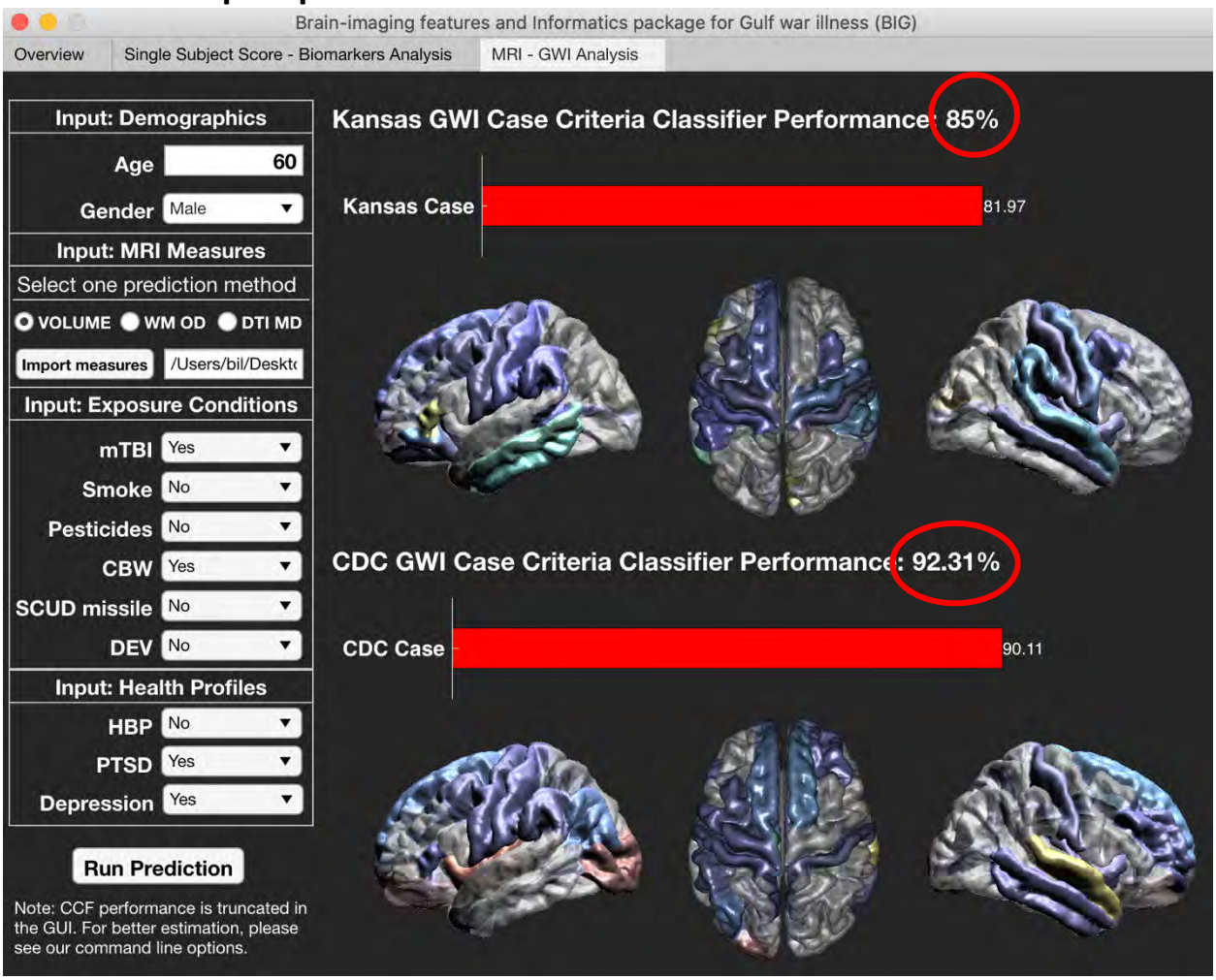
The current version provides GWI case status prediction for the Kansas GWI criteria and the CDC GWI criteria and displays the probability of GWI case status of the given subject. The prediction is calculated by a canonical correlation forest (CCF; Rainforth and Wood, 2015) model using subjects from GWIC.

The current version allows users to import 1 of the 3 brain imaging measures: T1 volume, WM OD, or DTI mean diffusivity (MD). User imported measure should be a 1 (row) by n (column) (n = number of ROIs for the selected measure) Excel spreadsheet. Users can select a file by clicking the Import measures button.

Compatible brain imaging measures can be created by (1) our in-house image processing pipeline that will be available upon request, (2) T1 volume measures can be extracted by FreeSurfer T1 reconstruction pipeline, DTI MD measures can be extracted by FSL TBSS steps,

and WM OD measures can be created by using our in-house diffusion processing script (available upon request).

Machine learning classification for Gulf War Illness case prediction - Step 2: Run and interpret prediction results



Once users hit the "Run Prediction" button, the GUI will start running the prediction. A message box will pop up while the prediction is running.

The GUI displays the classifier accuracy (red circle), which is calculated by a 10-fold cross validation process. In specific, subject data available from our repository are randomly separated into training (9-folds) and testing (1-fold) sets, where the model was first trained using training set, and then tested on the testing set for estimating performance. This process is repeated 10 times and the average accuracy is calculated and reported. The bar graphs display the predicted GWI case status probability based on users' imported measures and selections. The 3D brain figures display the ROIs that are selected as significant features for the classifier.

Glossary

DTI: Diffusion tensor imaging
GM: Gray matter
GWI: Gulf War Illness
MFI: Multidimensional Fatigue Inventory
ND: Neurite density measure
OD: Orientation dispersion measure
RSN: Resting state functional network
WM: White matter

References

Benjamini Y and 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. doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Cheng CH, Koo BB, Calderazzo S, Quinn E, Aenll K, Steele L, Klimas N, Krengel M, Janulewicz P, Toomey R, et al. (2020). Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. Brain Behav. Immun. 89:281–290. doi:10.1016/j.bbi.2020.07.006.

Fischl B. (2012). FreeSurfer. NeuroImage. 62(2):774–781. doi:10.1016/j.neuroimage.2012.01.021.

Ji JL, Spronk M, Kulkarni K, Repovs G, Anticevic A, Cole MW (2019). Mapping the human brain's cortical-subcortical functional network organization. NeuroImage. 185:35–57. doi:10.1016/j.neuroimage.2018.10.006.

Rainforth T and Wood F. (2015). Canonical correlation forest. arXiv. arXiv:1507.05444.

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. doi.org/10.1093/aje/152.10.992.

HHS Public Access

Author manuscript

Brain Behav Immun. Author manuscript; available in PMC 2019 February 19.

Published in final edited form as:

Brain Behav Immun. 2018 January ; 67: 42–46. doi:10.1016/j.bbi.2017.08.003.

Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness

Bang-Bon Koo[a,*,1], **Lindsay T. Michalovicz**[b,1], **Samantha Calderazzo**[a], **Kimberly A. Kelly**[b], **Kimberly Sullivan**[c], **Ronald J. Killiany**[a], and **James P. O'Callaghan**[b]

[a]School of Medicine, Boston University, Boston, MA, USA

[b]Health Effects Laboratory Division, Center for Disease Control and Prevention – National Institute for Occupational Safety and Health, Morgantown, WV, USA

[c]School of Public Health, Boston University, Boston, MA, USA

Abstract

Veterans of the 1991 Gulf War were potentially exposed to a variety of toxic chemicals, including sarin nerve agent and pesticides, which have been suspected to be involved in the development of Gulf War Illness (GWI). Several of these exposures cause a neuroinflammatory response in mice, which may serve as a basis for the sickness behavior-like symptoms seen in veterans with GWI. Furthermore, conditions mimicking the physiological stress experienced during the war can exacerbate this effect. While neuroinflammation has been observed post-exposure using animal models, it remains a challenge to evaluate neuroinflammation and its associated cellular and molecular changes *in vivo* in veterans with GWI. Here, we evaluated neuroimmune-associated alterations in intact brains, applying our existing GWI mouse model to rats, by exposing them to 4 days of corticosterone (CORT; 200 mg/L in the drinking water), to mimic high physiological stress, followed by a single injection of the sarin nerve agent surrogate, diisopropyl fluorophosphate (DFP; 1.5 mg/kg, i.p.). Then, we evaluated the neuroinflammatory responses using qPCR of cytokine mRNA and also examined brain structure with a novel high-order diffusion MRI. We found a CORT-enhancement of DFP-induced neuroinflammation, extending our mouse GWI model to the rat. High order diffusion MRI revealed different patterns among the different treatment groups. Particularly, while the CORT + DFP rats had more restricted spatial patterns in the hippocampus and the hypothalamus, the highest and most wide-spread differences were shown in DFP-treated rats compared to the controls in the thalamus, the amygdala, the piriform cortex and the ventral tegmental area. The association of these diffusion changes with neuroinflammatory cytokine expression indicates the potential for GW-relevant exposures to result in connectivity changes in the brain. By transferring this high order diffusion MRI into *in vivo* imaging in veterans with GWI, we can achieve further insights on the trajectories of the neuroimmune response over time and its impacts on behavior and potential neurological damage.

[*]Corresponding author at: Anatomy and Neurobiology, Boston University School of Medicine, Boston, MA 02118, USA. bbkoo@bu.edu (B.-B. Koo).

[1]These authors contributed equally to the work.

Keywords

Neuroimmune response; Diffusion MRI; Neuroinflammation; Gulf War Illness; Rat model; Cytokine; Corticosterone; Diisopropyl fluorophosphate; Rat model

1. Introduction

More than 25 years after the 1991 Gulf War, nearly one-third of the 697,000 U.S. troops who served continue to suffer from a complex, multi-symptom illness that is not well-explained by established medical or psychiatric diagnoses (White et al., 2016). The similarity of the symptoms associated with Gulf War Illness (GWI) to the classic symptoms of sickness behavior, including fatigue, chronic pain, memory complaints, and headaches, has high-lighted the possibility for GWI to be driven by underlying neuroinflammation (Dantzer and Kelley (2007)).

DoD modeling estimates 100,000 U.S. troops were potentially exposed to low level sarin and studies have found a potential impact of sarin in veterans with GWI (White et al., 2016). Accordingly, we developed a GWI mouse model incorporating exogenous corticosterone (CORT), to mimic physiological stress, and acute exposure to diisopropyl fluorophosphate (DFP), to mimic sarin nerve agent exposure experienced by GW veterans. This paradigm resulted in a marked brain-wide neuroinflammatory response in the absence of evidence of brain damage (O'Callaghan et al., 2015), highlighting the potential for these exposures to contribute to an underlying neuroinflammatory condition in GWI. In addition to mimicking sarin, DFP shares chemical characteristics with other irreversible acetylcholinesterase, organophosphate compounds that veterans were exposed to during the Gulf War, like the insecticides chlorpyrifos and dichlorvos. As such, we have demonstrated the potential for both DFP, as a sarin surrogate, and chlorpyrifos to instigate similar neuroinflammatory responses following CORT pretreatment (Locker et al., 2017), supporting a role for these classes of compounds in the development of GWI.

To draw an in-depth relationship between altered neuroinflammatory response and sickness behavior shown in veterans with GWI, it is important to have a monitoring method which is minimally invasive and allows for the combined evaluation of immuno-logical and neurological consequences of the toxic insults. Currently, there are different ways to examine neuroinflammatory responses *in vivo*. Measuring cytokine levels in CSF (Lenzlinger et al., 2004) may allow for the identification of proinflammatory markers in GWI. Also, positron emission tomography (PET) imaging can offer brain physiological information in GWI (Yehuda et al., 2010). However, the high invasiveness of lumbar puncture to obtain CSF and the costliness and insensitivity of PET (e.g., see Vivash and O'Brien, 2016) creates limits to the usefulness of these methods to address neuroinflammation in veterans with GWI. In order to bridge the animal to human extrapolation gap, magnetic resonance imaging (MRI) has been used to assess neural structural changes in association with an altered immune response, such as that proposed to underlie GWI. Morphometric MRI analysis of veterans with GWI confirmed overall reduction in the grey matter (GM) (Chao et al., 2010) and white matter (WM) (Heaton et al.,

2007; Chao et al., 2011), as well as a reduction in hippocampal and cortical GM volumes compared to healthy veterans (Chao et al., 2010). These regional morphometric changes have been tied to alterations in brain connectivity using diffusion MRI (Rayhan et al., 2013, Chao et al., 2011). Higher axial diffusivity measures have been reported in some WM major fiber pathways in the brains of GWI-suffering veterans. These findings may indicate that there are focal spots primarily involved in illness propagation in the brain; specifically, diffusion mapping may identify underlying structural and connectivity changes between brain cells (Anwander et al., 2010; McNab et al., 2013; Leuze et al., 2012).

2. Materials and methods

In this study, we expanded our established GWI mouse model (O'Callaghan et al., 2015; Locker et al., 2017) by exposing adult male Sprague Dawley rats (Hilltop Lab Animals, Scottdale, PA, USA) to CORT in the drinking water (200 mg/L in 0.6% EtOH) for 4 days, followed by a single injection of DFP (1.5 mg/kg, i.p.). Rats were sacrificed 6 h post-DFP by either decapitation for the evaluation of cortex cytokine mRNA (n = 5 rats/group), or by a fatal dose of pentobarbital-based euthanasia solution (Fatal Plus; 300 mg/kg, i.p.) followed by transcardial perfusion with 0.9% saline and fixation with 4% paraformaldehyde (n = 5 rats/group) for MRI. Total RNA from the frontal region of one cortical hemisphere was isolated as previously described (Locker et al., 2017). Real-time PCR analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and of the proinflammatory mediators, TNFα, IL-6, CCL2, IL-1β, leukemia inhibitor factor (LIF), and oncostatin M (OSM) was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in combination with TaqMan® chemistry as previously described (Locker et al., 2017). Relative quantification of gene expression was performed using the comparative threshold ($\Delta\Delta C_T$) method. Changes in mRNA expression levels were calculated after normalization to GAPDH. The ratios obtained after normalization are expressed as fold change over corresponding saline-treated con- trols and two-way ANOVAs (pretreatment [water or CORT]× exposure [saline or DFP]; $p \leq 0.05$) were conducted on log transformed values using SigmaPlot v12.5 (Systat Software, Inc., San Jose, CA, USA). If statistical significance was detected between groups by two-way ANOVA, then Bonferroni post hoc analysis (statistical significance, $p \leq 0.05$) was performed to evaluate the statistical significance of all pairwise multiple comparisons. For diffusion MRI imaging analysis, paraformaldehyde-perfused brains were scanned for 10 h on a 4.7 T Bruker MRI with an applied diffusion weighted spin-echo echo planar imaging sequence (SE-EPI) with the following parameters: 500 lm isotropic voxel, coronal slice acquisition with 515 independent diffusion gradient directions using b-values up to 40,000 s/mm^2 (Wedeen et al., 2008). For each brain, five non-diffusion weighted (b0) images were averaged to perform pre-processing of the raw diffusion scans and a modified in-house processing pipeline (Koo et al., 2013) was used to perform sequential pre-processing steps on the data. Q-space imaging method (Yeh et al., 2010) were used for the reconstruction of diffusion parameters. Three dimensional probability information on the diffusion displacement was calculated in each voxel in the brain scans and then formed the spin distribution function. Micro-scale diffusivity was modeled for partial diffusion encoding length based on the weighted sum of partial spin distributions below upper bound of the diffusion displacement (Yeh et al., 2016).

In this study, we applied 2 μm, 10 μm and 20 μm upper bounds for calculating the micro-scale diffusivity maps. We also calculated generalized fractional anisotropy (GFA) based on the spin distribution in q-space reconstruction. GFA has been used for quantifying microstructural integrity (similar to fractional anisotropy in diffusion tensor imaging) for q-space diffusion imaging. From the pre-processing, all diffusion indices on each brain were nonlinearly transformed to the atlas space to perform group-level statistics and all brain images were smoothed based on the Gaussian kernel (1.5 mm FWHM). Regional impacts were highlighted using unpaired group statistics between controls and the other groups (CORT, DFP, CORT+DFP) per each diffusion index separately. We conducted permutation-based random effect corrections for multiple comparison corrections with 5000 permutations to correct for possible random effects. Voxels with significant differences were defined by $p < 0.05$ and significant clusters were then mapped to the atlas to confirm anatomical information.

3. Results

Similar to our mouse model (O'Callaghan et al., 2015), we found that not only does DFP alone increase the expression of several of the evaluated genes, but also that prior CORT exposure significantly exacerbated this response (Fig. 1). The micro-scale diffusivity mapping successfully differentiated either CORT and/or DFP responses in the brain (Fig. 2A, first row) with higher micro-scale diffusivity values in the CORT, DFP, and CORT+DFP groups compared to controls (Fig. 2B). Among the diffusion upper bounds evaluated (Fig. 2A, second row), the 10 lm partial diffusion encoding revealed more statistically significant group differences, identifying more widespread clusters covering the hippocampus and outer cortices in the CORT+DFP group over controls. Furthermore, while the CORT+DFP treated brains had more restricted patterns in the hippocampus and the hypothalamus, the highest and most widespread differences were shown in the thalamus, amygdala, piri-form cortex and ventral tegmental area of DFP-treated (corrected $p < 0.001$) followed by CORT-treated (corrected $p<0.01$) rats. Generalized fractional anisotropy (GFA) revealed less significant differences compared to the micro-scale diffusivity measures. Differences between the controls and DFP-treated brains had spatial pattern overlap with the micro-scale diffusivity mapping results in regions including the medial frontal and hippocampal regions (Fig. 2 third row), whereas CORT and CORT+DFP had distinct patterns compared to their micro-scale mapping results.

4. Discussion

Here, we have shown that the CORT-enhanced DFP-induced neuroinflammatory model developed for the mouse (O'Callaghan et al., 2015) can be extended to the rat and that diffusion MRI can successfully differentiate between the exposure conditions of this GWI model. We have demonstrated previously that our model of GWI is an instigation of neuroinflammation without evidence of brain damage. This has been shown previously with immunohisto-chemistry at relevant, short-term time points in mice (O'Callaghan et al., 2015) through an absence of positive silver stain and Fluoro-Jade markers for degenerating neurons, as well as a lack of changes in microglia or astrocytes. Similar results have been confirmed in our rat model at comparable time points (unpublished data). Therefore, at this

early time point, we have shown the neuroinflammatory potential of DFP and CORT+DFP exposure in the absence of damage. Furthermore, what we have demonstrated here is that this neuroinflammatory response results in subtle, but differentiable changes in diffusion MRI, which highlights the ability to detect inflammatory-induced changes in MRI patterns early and without the requirement of severe damage to the brain tissue. Recent studies using similar diffusion parameters also reported successful discrimination of microstructural changes in neuronal or glial cell elements (Johnson et al., 2014, Blumenfeld-Katzir et al., 2011). In addition, high-order diffusion MRI successfully captured statistically significant changes in diffusion indices in a rat model of mild traumatic brain injury as early as 2 h post-injury (Zhuo et al., 2012). While the current study evaluates early time points in relation to a chronic illness, these conditions model what we would hypothesize to have been experienced immediately following exposure in theater or the proposed initiating events of GWI. Various studies of long-term time points that are more closely relevant to the current condition of veterans with GWI are on-going in our rodent models of GWI. Future studies will aim to address the potential for this model of “in theater” exposure conditions to progress into the chronic condition we associate with GWI and evaluate how these early diffusion changes may correlate or predict MRI results in a long-term model of GWI.

By using higher-order diffusion MRI, the limitations in the ability to assess minor changes in subcellular components typically associated with clinical diffusion MRI (typically, around $b = 1000$ s/mm^2) can be avoided (Palacios et al., 2014, Wang et al., 2015). Furthermore, the addition of GFA mapping, which is a common index for assessing the intactness of white matter tracts, revealed different patterns than the micro-scale assessments, indicating that exposure to DFP may affect micro-structural changes in major cortical connections. While more work is needed to understand how these patterns directly correlate to neuroinflammation, the identification of these unique MRI patterns in our GWI rodent model indicates the potential for underlying neuroinflammation to be associated with morphological and/or connectivity changes in neurons and glia. Considering that published and preliminary immunohistochemistry studies in both mice (O’Callaghan et al., 2015) and rats (unpublished data) have indicated no major, macroscopic changes indicative of cell death or morphological alterations of neurons or glia, we would hypothesize that these diffusivity changes are the result of more microscale changes in morphology like the arborization of dendrites or glial processes. These would be subtle changes in response to neuroinflammation that do not result in traditional, damage-induced “activation” of either microglia or astrocytes. Overall, since the high-order diffusion imaging protocol is also available to 3T clinical scanners, these results help to establish high-order diffusion MRI as a means to evaluate subtle ultra-structural changes in neural cells, which may be associated with *in vivo* neuroinflammation, in veterans with GWI.

Acknowledgments

Drs. Koo and Michalovicz contributed equally to this work. We appreciate the excellent technical assistance provided by Samantha Calderazzo, Brenda K. Billig and Christopher M. Felton. This work is supported by a department of Defense CDMRP GWI consortium award (W81XWH-13-2-0072). Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health. This work was supported by the Assistant Secretary of Defense for Health Affairs, through the Gulf War Illness Research Program, GW120037. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

References

Anwander A, Pampel A, Knosche TR, 2010 In vivo measurement of cortical anisotropy by diffusion weighted imaging correlates with cortex type. Proc. Int. Soc. Magn. Reson. Med 18, 109.

Blumenfeld-Katzir T, Pasternak O, Dagan M, Assaf Y, 2011 Diffusion MRI of structural brain plasticity induced by a learning and memory task. PLoS ONE 6 (6), e20678. [PubMed: 21701690]

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. NeuroToxicol. 32 (6), 814–822.

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. NeuroToxicol. 31 (5), 493–501. 10.1016/j.neuro.2010.05.006.

Dantzer R, Kelley KW, 2007 Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun 21 (2), 153–160. [PubMed: 17088043]

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 (4), 761–769. [PubMed: 17485118]

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]

Koo B-B, Oblak AL, Zhao Y, Farris CW, Bowley B, Rosene DL, Killiany RJ, 2013 Hippocampal network connections account for differences in memory performance in the middle-aged rhesus monkey. Hippocampus 23 (12), 1179–1188. [PubMed: 23780752]

Lenzlinger PM, Hans VHJ, Joller-Jemelka HI, Trentz O, Morganti-Kossmann MC, Kossmann C, 2004 Markers for cell-mediated immune response are elevated in cerebrospinal fluid and serum after severe traumatic brain injury in humans. J. Neurotrauma 18 (5), 479–489.

Leuze CW, Anwander A, Bazin PL, Stüber C, Reimann K, Geyer S, Turner R, 2012 Layer specific intracortical connectivity revealed with diffusion MRI. Cereb. Cortex 24 (2), 328–339. [PubMed: 23099298]

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. Neuorchem 10.1111/jnc.14071.

McNab JA, Polimeni JR, Wang R, Augustinack JC, Fujimoto K, Stevens A, Janssens T, Farivar R, Folkerth RD, Vanduffel W, Wald LL, 2013 Surface based analysis of diffusion orientation for identifying architectonic domains in the in vivo human cortex. NeuroImage 69, 87–100. [PubMed: 23247190]

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 (5), 708–721. [PubMed: 25753028]

Palacios RDY, Verhoye M, Henningsen K, Wiborg O, Van der Linden A, 2014 Diffusion kurtosis imaging and high-resolution MRI demonstrate structural aberrations of caudate putamen and amygdala after chronic mild stress. PLoS ONE 9 (4), e95077. [PubMed: 24740310]

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. [PubMed: 23526988]

Vivash L, O'Brien TJ, 2016 Imaging microglial activation with TSPO PET: lighting up neurologic diseases? J. Nucl. Med 57 (2), 165–168. [PubMed: 26697963]

Wang Y, Sun P, Wang Q, Trinkaus K, Schmidt RE, Naismith RT, et al., 2015 Differentiation and quantification of inflammation, demyelination and axon injury or loss in multiple sclerosis. Brain 138 (5), 1223–1238. [PubMed: 25724201]

Wedeen R, Wang J, Schmahmann T, Benner W, Tseng G, Dai D, Pandya P, Hagmann Arceuil H de Crespigny A, 2008 Diffusion spectrum magnetic resonance imaging (DSI) tractography of crossing fibers. Neuroimage 41 (4), 1267–1277. [PubMed: 18495497]

White RF, Steele L, O'Callaghan JP, Sullivan K, Binns JH, Golomb BA, et al., 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]

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, 2016 Mapping immune cell infiltration using restricted diffusion MRI. Magn. Reson. Med 77 (2), 603–612. [PubMed: 26843524]

Yehuda R, Golier JA, Bierer LM, Mikhno A, Pratchett LC, Burton CL, et al., 2010 Hydrocortisone responsiveness in Gulf War veterans with PTSD: effects on ACTH, declarative memory hippocampal [18F]FDG uptake on PET. Psychiatry Res. 184 (2), 117–127. [PubMed: 20934312]

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]

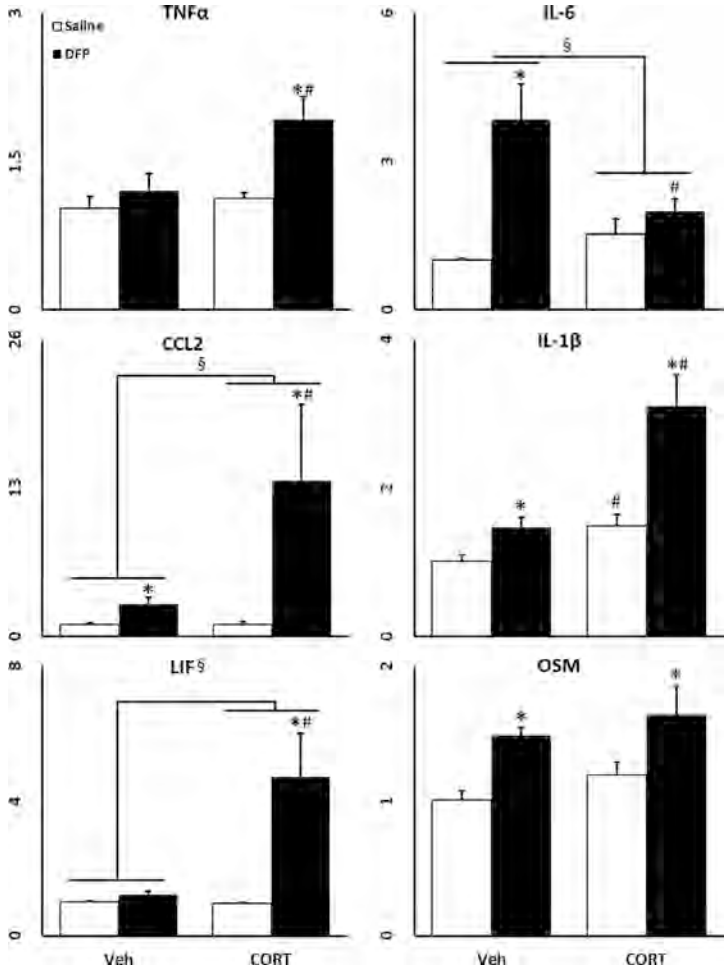


Fig. 1.

Chronic CORT exacerbates DFP induced inflammation in rats. Effects of chronic CORT pretreatment (200 mg/L 0.6% EtOH in drinking water for 4 days) on DFP (1.5 mg/kg, i.p.) induced neuroinflammation in the cortex at 6 h post DFP exposure is shown with TNFα, IL-6, CCL2, IL-1β, LIF, and OSM qPCR. Data represents mean ± SEM (n = 5 rats/group). Statistical significance of at least $p < 0.05$ is denoted by * as compared within pretreatment (vehicle or CORT), # as compared within exposure (saline or DFP), and § for a significant interaction between pretreatment and exposure.

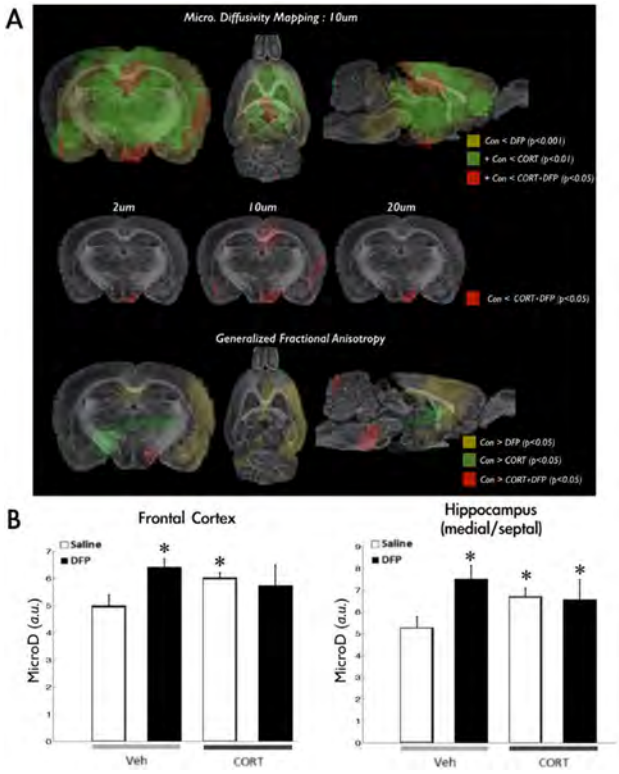


Fig. 2.

Microscale diffusivity mapping in CORT + DFP treated rats. Group differences in microscale diffusivity is shown in panel A. Statistically significant differences between controls and DFP treated rats are shown in the clusters with yellow color encodings. Green clusters are overlapped to the yellow clusters and indicates differences between controls and CORT-treated rats. Red clusters are overlapped to the previous two clusters and show differences between controls and CORT + DFP treated rats. We confirmed that diffusion encoding length at 10 lm had more sensitivity to detect statistically significant group differences in between controls and CORT + DFP treated rats as shown in the second row of panel A. Generalized fractional anisotropy maps revealed distinct patterns with lower statistical thresholds compared to the micro diffusivity maps (third row in panel A). Panel B shows quantification of micro diffusivities in the frontal cortex and the hippocampus. In both graphs, white and black bar in Veh section shows average micro-diffusivity value of each region of interest in saline and DFP accordingly. Also, white and black bar in CORT section shows average micro-diffusivity value of each region of interest in CORT and CORT + DFP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Brain, Behavior, and Immunity 89 (2020) 281–290

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

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

Chia-Hsin Cheng[a,1], Bang-Bon Koo[a,*,1], Samantha Calderazzo[a], Emily Quinn[b], Kristina Aenlle[c,d], Lea Steele[e], Nancy Klimas[f], Maxine Krengel[b], Patricia Janulewicz[b], Rosemary Toomey[b], Lindsay T. Michalovicz[g], Kimberly A Kelly[g], Timothy Heeren[b], Deborah Little[h], James P. O'Callaghan[g], Kimberly Sullivan[b,*]

[a] School of Medicine, Boston University, Boston, MA, USA
[b] School of Public Health, Boston University, Boston MA USA
[c] Nova Southeastern University, Fort Lauderdale, FL, USA
[d] Department of Veterans Affairs, Miami VA Healthcare System, Research Service, Miami, FL, USA
[e] Baylor College of Medicine, Houston, TX, USA
[f] Nova Southeastern University, Fort Lauderdale, FL, USA
[g] Health Effects Laboratory Division, Center for Disease Control and Prevention – National Institute for Occupational Safety and Health, Morgantown, WV, USA
[h] University of Texas, Houston, TX, USA

ARTICLE INFO

Keywords:
Gulf War Illness
Veterans
Cytokines
Neuroinflammation
Diffusion imaging
MRI

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 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.

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

* Corresponding authors.
E-mail address: bbkoo@bu.edu (B.-B. Koo).
[1] co-1st authors.

https://doi.org/10.1016/j.bbi.2020.07.006
Received 6 March 2020; Received in revised form 11 June 2020; Accepted 8 July 2020
Available online 31 July 2020

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).

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%

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).

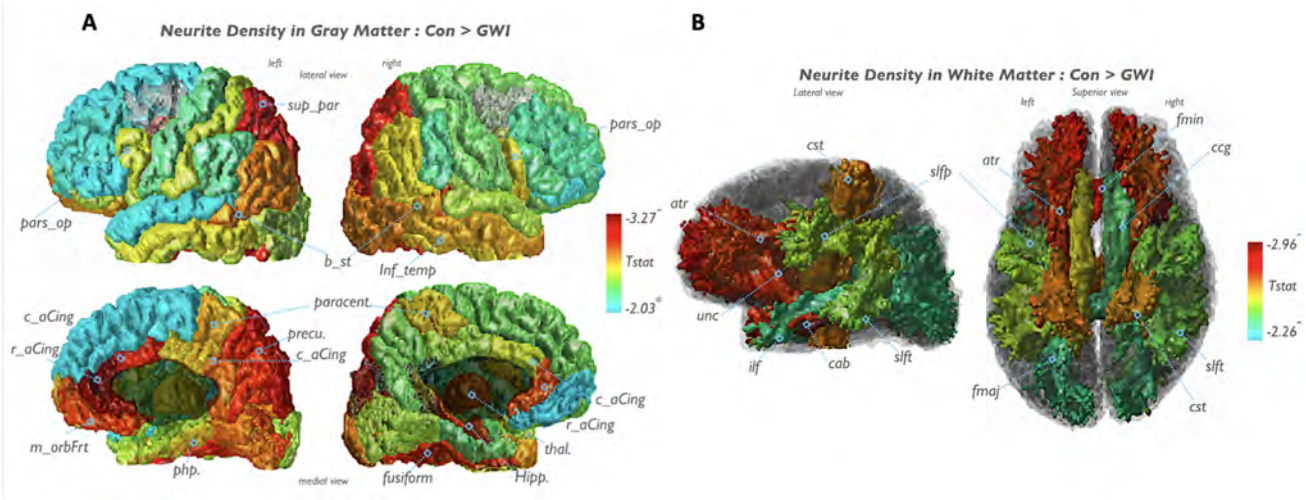


Fig. 1. ND feature mapping of whole group WM and GM analyses highlights group effects in widespread regions, most significantly seen in frontal white matter tracts and subcortical limbic regions. Fmaj = corpus callosum forceps major, fmin = corpus callosum forceps minor, atr = anterior thalamic radiations, 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.

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

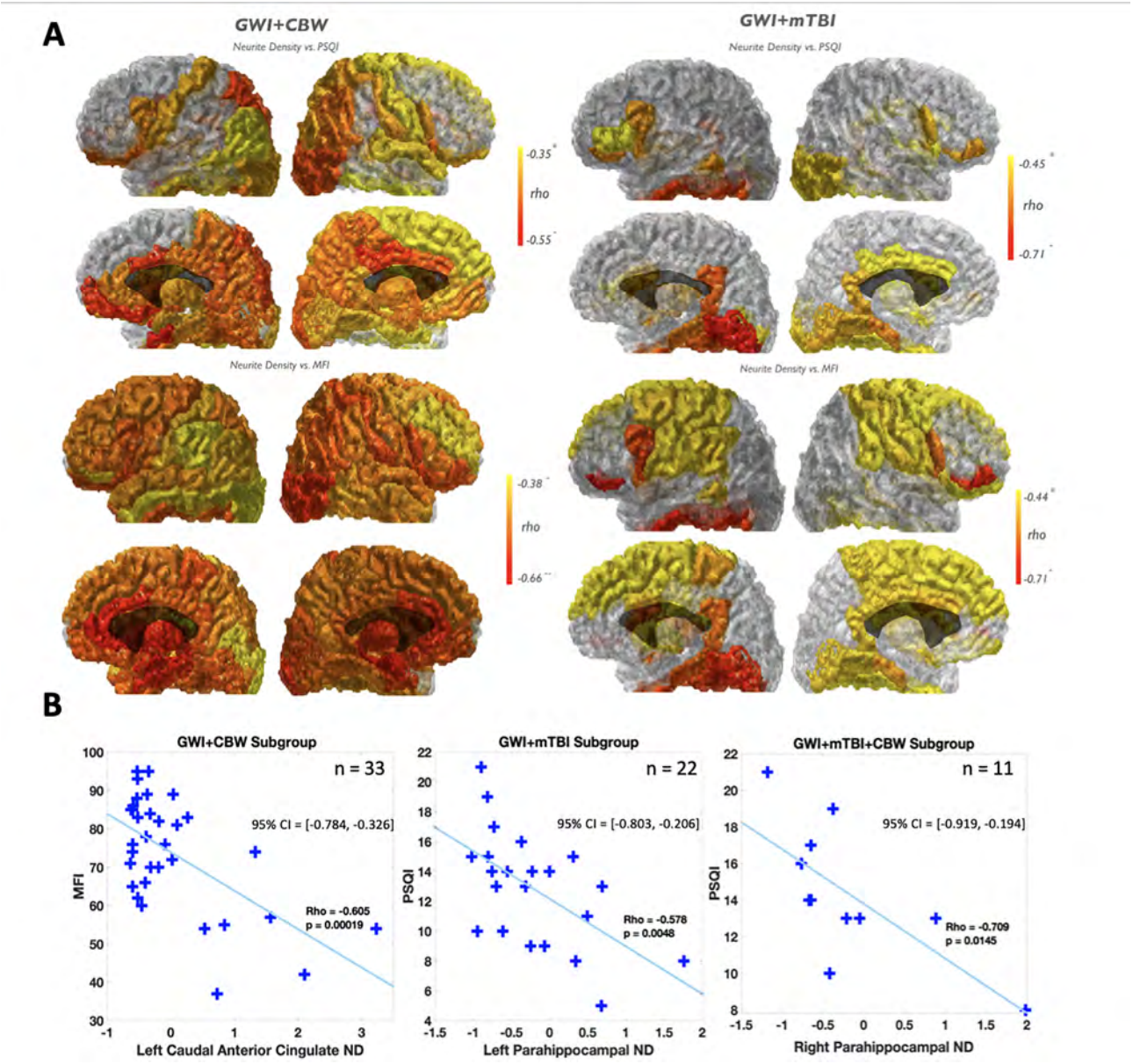


Fig. 2. Self-reported symptoms correlation mapping in GWI subjects exposed to chemical or biological warfare agents or mTBI. Regions with significant correlation between ND and PSQI (A, left upper) or MFI (A, left lower) in GW veterans with chemical/biological warfare agent exposures are rendered based 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.

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

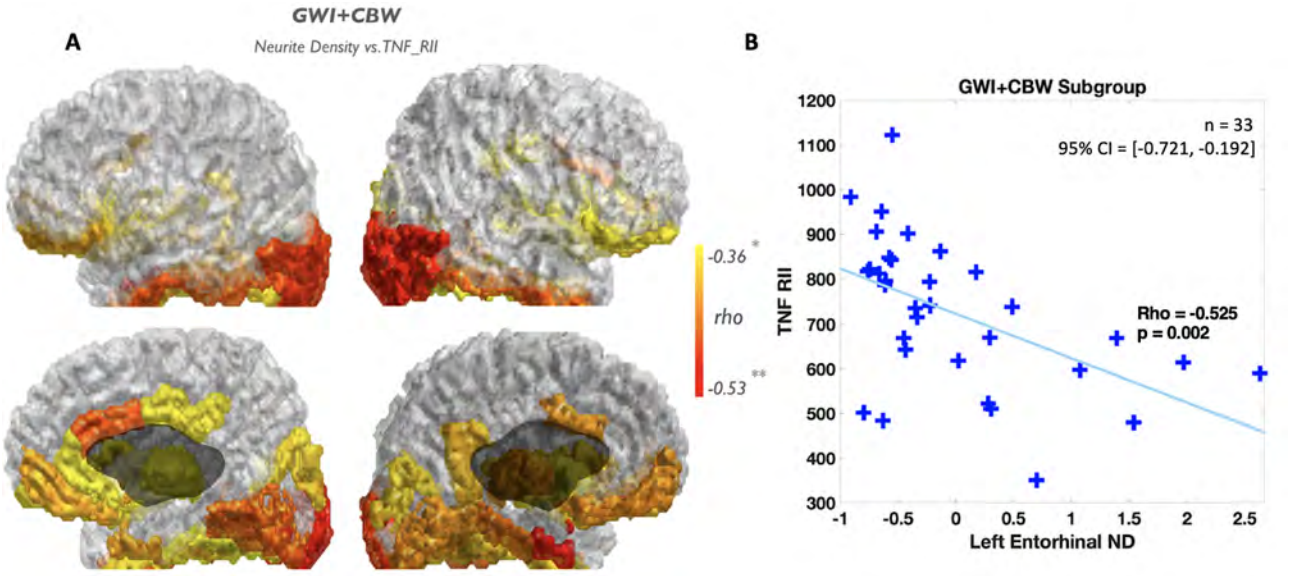


Fig. 3. Blood cytokine correlation mapping in GWI subjects exposed to chemical and biological warfare agents. Regions with significant correlation between ND and TNF_RII (A) are rendered based on significance levels. Panel B shows data distribution patterns of ND and TNF_RII levels in the representative region within the GWI + CBW subgroup. * $p < 0.05$, ** $p < 0.01$.

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).

Acknowledgements

Ms. Cheng and Dr. Koo contributed equally to this work as the 1st author. Drs. Koo and Sullivan are the corresponding authors. This work is supported by a department of Defense CDMRP new investigator award (W81XWH-17-1-0440) and CDMRP GWI consortium award (W81XWH-13-2-0072).

Disclaimer

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. This work was supported by the Assistant Secretary of Defense for Health Affairs, through the Gulf War Illness Research Program, GW160032 and GW120037. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

Appendix A. Supplementary data

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

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, M.B., Conboy, L.A., Kokkotou, E., Jacobson, E., Elmasry, E.M., Elkafrawy, P., Neely, M., Bass, C.R., Sullivan, K., 2017. Screening for novel central nervous system biomarkers in veterans with Gulf War Illness. Neurotoxicol. Teratol. 61, 36–46.

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

Banks, C.N., Lein, P.J., 2012. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology 33 (3), 575–584.

Barnes, D.E., Byers, A.L., Gardner, R.C., Seal, K.H., Boscardin, W.J., 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.

Belgrad, J., Dutta, D.J., Bromley-Coolidge, S., Kelly, K.A., Michalovicz, L.T., Sullivan, K.A., O'Callaghan, J.P., Fields, R.D., 2019. Oligodendrocyte involvement in Gulf War Illness. Glia 67 (11), 2107–2124.

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, H.M., Dietrich, W.D., 2002. Quantitative changes in white and gray matter 1 year following traumatic brain injuries in rats. Acta Neuropathol. 103, 607–614.

Broderick, G., Kreitz, A., Fuite, J., Fletcher, M.A., Vernon, S.D., Klimas, N., 2011. A pilot study of immune network remodeling under challenge in Gulf War Illness. Brain Behav. Immun. 25 (2), 302–313.

Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. Psychiatry Res. 28, 193–213.

Chao, L.L., Rothlind, J.C., Cardenas, V.A., Meyerhoff, D.J., Weiner, M.W., 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.

Chao, L.L., Abadjian, L., Hlavin, J., Meyerhoff, D.J., Weiner, M.W., 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.

Chao, L.L., 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.

Chao, L.L., 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.

Colgan, N., Siow, B., O'Callaghan, J.M., Harrison, I.F., Wells, J.A., Holmes, H.E., Ismail, O., Richardson, S., Alexander, D.C., Collins, E.C., Fisher, E.M., Johnson, R., Schwarz, A.J., Ahmed, Z., O'Neill, M.J., Murray, T.K., Zhang, H., Lythgoe, M.F., 2016. Application of neurite orientation dispersion and density imaging (NODDI) to a tau pathology model of Alzheimer's disease. NeuroImage 125, 739–744.

Desikan, R.S., Ségonne, F., Fischl, B., Quinn, B.T., Dickerson, B.C., Blacker, D., Buckner, R.L., Dale, A.M., Maguire, R.P., Hyman, B.T., Albert, M.S., Killiany, R.J., 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.

Fischl, B., 2012. FreeSurfer. NeuroImage 62 (2), 774–781.

Fletcher, M.A., Zeng, X.R., Barnes, Z., Levis, S., Klimas, N.G., 2009. Plasma cytokines in women with chronic fatigue syndrome. J. Transl. Med. 7, 96.

Fukutomi, H., Glasser, M.F., Zhang, H., Autio, J.A., Coalson, T.S., Okada, T., Togashi, K., Van Essen, D.C., Hayashi, T., 2018. Neurite imaging reveals microstructural variations in human cerebral cortical gray matter. NeuroImage 182 (15), 488–499.

Gade, D.M., Wenger, J.B., 2011. Combat exposure and mental health: the long-term effects among US Vietnam and Gulf War veterans. Health Econ. 20, 401–416.

Glasser, M.F., Goyal, M.S., Preuss, T.M., Raichle, M.E., Van Essen, D.C., 2014. Trends and properties of human cerebral cortex: Correlations with cortical myelin content. NeuroImage 93 (part. 2), 165–175.

Grussu, F., Schneider, T., Tur, C., Yates, R.L., Tachrount, M., Ianus, A., Yiannakas, M.C., Newcombe, J., Zhang, H., Alexander, D.C., DeLuca, G.C., Gandini Wheeler-Kingshott, C.A.M., 2017. Neurite dispersion: a new marker of multiple sclerosis spinal cord pathology? Ann. Clin. Transl. Neurol. 4 (9), 663–679.

Golomb, B.A., 2008. Acetylcholinesterase inhibitors and Gulf War illnesses. Proc. Natl. Acad. Sci. 105 (11), 4295–4300.

Groppe, D.M., Urbach, T.P., 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.

Gur, R.C., Mozley, P.D., Resnick, S.M., Gottlieb, G.L., 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.

Hawari, F.I., Rouhani, F.N., Cui, X., Yu, Z.X., Buckley, C., Kaler, M., Levine, S.J., 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.

Heaton, K.J., Palumbo, C.L., Proctor, S.P., Killiany, R.J., Yurgelun-Todd, D.A., White, R.F., 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.

Hoge, C.W., McGurk, D., Thomas, J.L., Cox, A.L., Engel, C.C., Castro, C.A., 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.

Janulewicz, P.A., Seth, R.K., Carlson, J.M., Ajama, J., Quinn, E., Heeren, T., Klimas, N., Lasley, S.M., Horner, R.D., 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, G.E., Klimas, N., Broderick, G., Craddock, T.J.A., 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, M.G., Krengel, M., Kibler, J.L., Zundel, C., Klimas, N.G., Sullivan, K., Craddock, T.J.A., 2019. Neuropsychological findings in Gulf War illness: a review. Front. Psychol. 10, 2088.

Jenkinson, M., Beckmann, C.F., Behrens, T.E.J., Woolrich, M.W., Smith, S.M., 2012. FSL. NeuroImage 62, 782–790.

Johnson, G.A., Calabrese, E., Little, P.B., Hedlund, L., Qi, Y., Badea, A., 2014. Quantitative mapping of trimethyltin injury in the rat brain using magnetic resonance histology. Neurotoxicology 42, 12–23.

Khaiboullina, S.F., DeMeirleir, K.L., Rawat, S., Berk, G.S., Gaynor-Berk, R.S., Mijatovic, T., Blatt, N., Rizvanov, A.A., Young, S.G., Lombardi, V.C., 2014. Cytokine expression provides clues to the pathophysiology of Gulf War illness and myalgic encephalomyelitis. Cytokine 72 (1), 1–8.

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.

Koo, B.B., Michalovicz, L.T., Calderazzo, S., Kelly, K.A., Sullivan, K., Killiany, R.J., O'Callaghan, J.P., 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, D.J., 2012. Neuroinflammation after traumatic brain injury: opportunities for therapeutic intervention. Brain Behav. Immun. 26, 1191–1201.

Locker, A.R., Michalovicz, L.T., Kelly, K.A., Miller, J.V., Miller, D.B., O'Callaghan, J.P., 2017. Corticosterone primes the neuroinflammatory response to Gulf War Illness-relevant organophosphates independently of acetylcholinesterase inhibition. J. Neurochem. 142 (3), 444–455.

McCunn, P., Gilbert, K.M., Zeman, P., Li, A.X., Strong, M.J., Khan, A.R., 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.

Michalovicz, L.T., Locker, A.R., Kelly, K.A., Miller, J.A., Barnes, Z., Fletcher, M.A., Miller, D.B., Klimas, N.G., Morris, M., Lasley, S.M., O'Callaghan, J.P., 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.

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, J.V., LeBouf, R.F., Kelly, K.A., Michalovicz, L.T., Ranpara, A., Locker, A.R., Miller, D.B., O'Callaghan, J.P., 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.

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

Naughton, S.X., Hernandez, C.M., Beck, W.D., Poddar, I., Yanasak, N., Lin, P.C., Terry Jr., A.V., 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, J.P., Kelly, K.A., Locker, A.R., Miller, D.B., Lasley, S.M., 2015. Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. J. Neurochem. 133, 708–721.

O'Callaghan, J.P., Miller, D.B., 2019. Neuroinflammation disorders exacerbated by environmental stressors. Metabolism 100S, 153951.

O'Donovan, A., Chao, L.L., Paulson, J., Samuelson, K.W., Shigenaga, J.K., Grunfeld, C., Weiner, M.W., Neylan, T.C., 2015. Altered inflammatory activity associated with reduced hippocampal volume and more severe posttraumatic stress symptoms in Gulf War veterans. Psychoneuroendocrinology 51, 557–566.

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.

Proctor, S.P., Heaton, K.J., Heeren, T., White, R.F., 2006. Effects of sarin and cyclosarin exposure during the 1991 Gulf War on neurobehavioral functioning in US army veterans. NeuroToxicology 27 (6), 931–939.

Proctor, S.P, Heeren, T, White, R.F., Wolfe, J., Borgos, M.S., Davis, J.D., Pepper, L., Clapp, R., Sutker, P.B., Vasterling, J.J., 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.

Puri, B.K., Jakeman, P.M., Agour, M., Gunatilake, K.D.R., Fernando, K.A.C., Gurusinghe, A.I., Treasaden, I.H., Waldman, A.D., 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, L.L., Werner, A., Blüthmann, H., Doetschmann, T., Kreutzberg, G.W., 1999. Molecular signals for glial activation: Pro- and anti-inflammatory cytokines in the injured brain. Acta Neurochir. Suppl. 73, 21–30.

Rao, A.N., Patil, A., Brodnik, Z.D., Qiang, L., España, R.A., Sullivan, K.A., Black, M.M., Baas, P.W., 2017. Pharmacologically increasing microtubule acetylation corrects stress-exacerbated effects of organophosphates on neurons. Traffic 18 (7), 433–441.

Rathbone, A.T., Tharmaradinam, S., Jiang, S., Rathbone, M.P., Kumbhare, D.A., 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.

Rayhan, R.U., Stevens, B.W., Timbol, C.R., Adewuyi, O., Walitt, B., VanMeter, J.W., Baraniuk, J.N., 2013. Increased brain white matter axial diffusivity associated with fatigue, pain, and hyperalgesia in Gulf War illness. PLoS ONE 8 (3), e58493.

Robbins, C.A., Daneshevar, D.H., Picano, J.D., Gevett, B.E., Baugh, C.M., Riley, D.O., Nowinski, C.J., McKee, A.C., Cantu, R.C., Stern, R.A., 2014. Self-reported concussion history: impact of providing a definition of concussion. Open Access J. Sports Med. 7, 99–103.

Seichepine, D.R., Stamm, J.M., Daneshvar, D.H., Riley, D.O., Baugh, C.M., Gavett, B.E., Tripodis, Y., Martin, B., Chaisson, C., McKee, A.C., Cantu, R.A., Nowinski, C.J., Stern, R.A., 2013. Profile of self-reported problems with executive functioning in college and professional football players. J. Neurotrauma 30, 1299–1304.

Smets, E.M.A., Garssen, B., Bonke, B., De Haes, J.C.J.M., 1995. The Multidimensional Fatigue Inventory (MFI) Psychometric Qualities of an Instrument to Assess Fatigue. J. Psychosom. Res. 39 (5), 315–325.

Smith, D.H., Johnson, V.E., Stewart, W., 2013. Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nature Rev. Neurol. 9 (4), 211–221.

Spradling, K.D., Lumley, L.A., Robison, C.L., Meyerhoff, J.L., Dillman, J.F., 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.

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, T.A., Heeren, T., White, R.F., 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.

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

Tang, L.W., Zheng, H., Chen, L., Zhou, S.Y., Huang, W.J., 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, J.J., Baker, D.G., Reda, D.J., Lyons, M.J., Henderson, W.G., Kang, H.K., Eisen, S.A., Murphy, F.M., 2009. Neuropsychological functioning of U.S. Gulf War veterans 10 years after the war. J. Int.Neuropsychological Soc. 15 (5), 717–729.

Vynorius, K.C., Paquin, A.M., Seichepine, D.R., 2016. Lifetime multiple mild traumatic brain injuries are associated with cognitive and mood symptoms in young healthy college students. Front. Neurol. 7, 188.

Tuch, D.S., 2004. Q-ball Imaging. Magn. Reson. Med. 52 (6), 1358–1372.

Whistler, T., Fletcher, M.A., Lonergan, W., Zeng, X.R., Lin, J.M., LaPerriere, A., Vernon, S.D., Klimas, N.G., 2009. Impaired immune function in Gulf War Illness. BMC Med. Genomics 2, 12.

White, R.F., Steele, L., O'Callaghan, J.P., Sullivan, K., Binns, J.H., Golomb, B.A., Bloom, F.E., Bunker, J.A., Crawford, F., Graves, J.C., Hardie, A., Klimas, N., Knox, M., Meggs, W.J., Melling, J., Philbert, M.A., 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.

Winkler, A.M., Ridgway, G.R., Webster, M.A., Smith, S.M., Nichols, T.E., 2014. Permutation inference for the general linear model. NeuroImage 92, 381–397.

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.

Yee, M.K., Seichepine, D.R., Janulewicz, P.A., Sullivan, K.A., Proctor, S.P., Krengel, M.H., 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, M.K., Janulewicz, P.A., Seichepine, D.R., Sullivan, K.A., Proctor, S.P., Krengel, M.H., 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, F.C., Wedeen, V.J., Tseng, W.Y., 2010. Generalized q-Sampling Imaging. IEEE Trans. Med. Imaging 29 (9), 1626–1635.

Yeh, F.C., Liu, L., Hitchens, T.K., Wu, Y.L., 2017. Mapping immune cell infiltration using restricted diffusion MRI. Magn. Reson. Med. 77, 603–612.

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.
Zhang, H., Schneider, T., Wheeler-Kingshott, C.A., Alexander, D.C., 2012. NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. NeuroImage 61, 1000–1016.
Zhuo, J., Xu, S., Proctor, J.L., Mullins, R.J., Simon, J.Z., Fiskum, G., Gullapalli, R.P., 2012. Diffusion kurtosis as an in vivo imaging marker for reactive astrogliosis in traumatic brain injury. Neuroimage 59 (1), 467–477.
Zundel, C.G., Krengel, M.H., Heeren, T., Yee, M.K., Grasso, C.M., Janulewicz Lloyd, P.A., Coughlin, S.S., 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.

brain sciences

MDPI

Article

Neuroimaging Markers for Studying Gulf-War Illness: Single-Subject Level Analytical Method Based on Machine Learning

Yi Guan [1,†], Chia-Hsin Cheng [1,†], Weifan Chen [1], Yingqi Zhang [1], Sophia Koo [1], Maxine Krengel [1], Patricia Janulewicz [2], Rosemary Toomey [1], Ehwa Yang [3], Rafeeque Bhadelia [4], Lea Steele [5], Jae-Hun Kim [3], Kimberly Sullivan [2,*] and Bang-Bon Koo [1,*]

1 School of Medicine, Boston University, Boston, MA 02118, USA; guanyi1@bu.edu (Y.G.); chiahsin@bu.edu (C.-H.C.); wfchen@bu.edu (W.C.); yqz2019@bu.edu (Y.Z.); sskoo@bu.edu (S.K.); mhk@bu.edu (M.K.); toomey@bu.edu (R.T.)
2 School of Public Health, Boston University, Boston, MA 02118, USA; paj@bu.edu
3 Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Korea; ehwayang@gmail.com (E.Y.); jaehun1115.kim@samsung.com (J.-H.K.)
4 Department of Radiology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA; rbhadelia@gmail.com
5 Neuropsychiatry Division, Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, TX 77030, USA; Lea.Steele@bcm.edu
* Correspondence: tty@bu.edu (K.S.); bbkoo@bu.edu (B.-B.K.)
† Both authors contributed equally to this work.

Received: 28 September 2020; Accepted: 17 November 2020; Published: 20 November 2020

Abstract: Gulf War illness (GWI) refers to the multitude of chronic health symptoms, spanning from fatigue, musculoskeletal pain, and neurological complaints to respiratory, gastrointestinal, and dermatologic symptoms experienced by about 250,000 GW veterans who served in the 1991 Gulf War (GW). Longitudinal studies showed that the severity of these symptoms often remain unchanged even years after the GW, and these veterans with GWI continue to have poorer general health and increased chronic medical conditions than their non-deployed counterparts. For better management and treatment of this condition, there is an urgent need for developing objective biomarkers that can help with simple and accurate diagnosis of GWI. In this study, we applied multiple neuroimaging techniques, including T1-weighted magnetic resonance imaging (T1W-MRI), diffusion tensor imaging (DTI), and novel neurite density imaging (NDI) to perform both a group-level statistical comparison and a single-subject level machine learning (ML) analysis to identify diagnostic imaging features of GWI. Our results supported NDI as the most sensitive in defining GWI characteristics. In particular, our classifier trained with white matter NDI features achieved an accuracy of 90% and F-score of 0.941 for classifying GWI cases from controls after the cross-validation. These results are consistent with our previous study which suggests that NDI measures are sensitive to the microstructural and macrostructural changes in the brain of veterans with GWI, which can be valuable for designing better diagnosis method and treatment efficacy studies.

Keywords: Gulf War illness; MRI; objective biomarker; machine learning; Kansas case criteria; diffusion; grey matter; neurite density imaging

1. Introduction

Gulf War illness (GWI) refers to the variety of chronic symptoms experienced by about 250,000 United States veterans who served in the 1991 Gulf War (GW) [1]. According to the Kansas

case criteria, symptoms of GWI fall into six categories: fatigue (fatigue and sleep problems), pain (joint and muscle), neurological (cognitive, mood, headache, and dizziness), respiratory (persistent cough and wheezing), gastrointestinal (diarrhea and nausea), and skin (rashes and other) problems. Exposure to neurotoxicant chemicals (organophosphate pesticides and sarin) during the war and other central nervous system (CNS) damage, such as mild traumatic brain injury (mTBI), are thought to have caused an innate immune over-response in the CNS, resulting in the development of these chronic GWI symptoms [2–7]). In order to meet the Kansas criteria for GWI, veterans must display chronic symptoms in at least three of the six categories, without presenting concurrent psychiatric and medical disorders [8]. However, accurate diagnoses of GWI remained challenging due to the heterogeneous clinical presentation of this condition, as well as the level of subjectivity associated with self-reported symptoms and neurotoxicant exposure history [8–10]. To improve management and treatment of GWI, there is an urgent need for defining sensitive and objective biomarkers of the disorder.

Previous neuroimaging studies demonstrated distinct changes within brains of veterans with GWI, which may underlie physiological symptoms. For example, T1W-MRI studies showed that GW veterans with exposure to the neurotoxicant chemical sarin exhibit reduced gray matter (GM) and white matter (WM) volumes, as well as reductions in hippocampal subfield volumes when compared to non-exposed veterans [11,12]. More recent studies using diffusion tensor imaging (DTI) have shown greater hippocampal mean diffusivity (MD) and increased axial diffusivity (AD) in the WM of sarin and cyclosarin exposed GW veterans, which are correlated to fatigue, pain, or hyperalgesia, and may serve as a potential biomarker for GWI [13–15]. We have previously applied a novel MRI diffusion processing method, neurite density imaging (NDI), on high-order diffusion MRI to demonstrate that the NDI measure scan successfully identify and validate different levels of neurological abnormalities in veterans with GWI from the Boston Gulf War Illness Consortium cohort [16].

ML algorithms have been applied to study a wide range of neurological disorders, including Alzheimer's disease, Parkinson's disease, and traumatic brain injury [17,18]. These studies have reported promising results for identifying diagnostic biomarkers [19,20]. The ML approach have strengths on exploiting features from different domains (i.e., neuropsychological, genetic and neuroimaging) and providing further insights on the potential interactions between different markers for classifying illness [21]. For the current study, we aimed to expand our previous work (on NDI) to cross-compare different types of neuroimaging markers (T1W-MRI, DTI and NDI) to determine whether these measures are useful for single subject-level classification of GWI cases vs. controls. Specifically, we incorporated the machine learning (ML) framework to search out key imaging features valuable for defining GWI. Computerized models were then trained based on the selected features and tested for classifying veterans with GWI.

2. Methods

2.1. Participants

In this study, we included brain imaging data of 119 GW veterans from Boston University Gulf War Illness Consortium (GWIC) (Table 1). GWIC is a multi-site study designed to identify the etiology and potential biomarkers of GWI. The inclusion criterion was deployment to the GW between August 1990 and July 1991. The exclusion criteria included having a diagnosis of chronic medical illnesses that could otherwise account for the symptoms experienced by GW veterans, including autoimmune, CNS, or major psychiatric disorders that could affect the brain and immune functions (e.g., epilepsy, stroke, severe head injury, etc.). Each participant completed an assessment protocol of health surveys, a neuropsychological test battery, brain imaging, and collection of blood and saliva samples [2]. In this study, we utilized brain imaging outcomes to study GWI. All participants provided written informed consent to participate in the study. This study was reviewed and approved by the Boston University institutional review board.

Table 1. Subject Characteristics.

BU Subjects	GW Control	GWI Case
N	21	98
Age (years)	54.06	52.46
Gender (F/M)	3/18	20/78

Gulf War Illness Criteria and Symptom Surveys

GWI case status was defined from the Kansas GWI case definition, which requires 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 [8]. 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. GWIC subjects were administered a general demographic information and medical conditions questionnaire and the Kansas Gulf War and health questionnaire for assessing symptoms [8,10]. 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 suggested worse conditions [22–24].

2.2. Image Acquisition

All veterans were scanned on an Achieva 3T whole-body MRI scanner (Philips Healthcare, Best, The Netherlands) at the Center of Biomedical Imaging, Boston University school of Medicine. T1W-MRI were obtained using an MPRAGE sequence developed by the Alzheimer's disease neuroimaging initiative (ADNI) (Repetition time (TR) = 6.8 ms, Echo time (TE) = 3.1 ms, flip angle = 9°, slice thickness = 1.2 mm, 170 slices, Field of view (FOV) = 250 mm, matrix = 256 × 256) (accessible from http://adni.loni.usc.edu/). Diffusion MRI data were obtained using 124 gradient directions utilizing parallel imaging on a 16-channel parallel head coil (70 slices, TR = 13,214 ms, TE = 55 ms, with a matrix size of 128 × 128 yielding a resolution of 2.0 × 2.0 × 2.0 mm^3, no slice gap). Multi-shell diffusion encodings with b-values 1000, 2000 and 3000 s/mm^2 were acquired with a single-shot echo planar imaging (EPI) sequence, and 6 b = 0 s/mm^2 field maps were collected in addition to distortion corrections built into the scanner.

2.3. Image Processing and Anatomical Defining

Structural T1W-MRI scans were analyzed with the Freesurfer package (version 6.0) to generate anatomical regions of interest (ROI) for assessing GM morphometric measures, and to provide GM anatomical co-registration references for diffusion images [25]. A total of 78 ROIs defined in the average template space were co-registered to each subject's cortical surface by applying nonlinear co-registration parameters. All results were visually inspected for artifacts or incomplete segmentation. Fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) maps were created using tract-based spatial statistics (TBSS), part of FSL package that projects all subjects' diffusion tensor imaging (DTI) data onto a mean tract skeleton [26]. A total of 20 major WM tracts were defined using the Johns Hopkins University (JHU) white-matter tractography atlas provided in the FSL package, the same template was also used for special normalization and linear co-registration of diffusion MRIs [27,28].

2.4. High-Order Diffusion Processing

Microstructural diffusion measures were reconstructed from multi-shell diffusion MRI images containing 3 b-value encodings using the NDI model [16]. Two parameters, neurite density (ND)

index and orientation dispersion (OD) index were extracted from the NDI model. In brief, ND is a fraction of tissue composed of neurites which include axons and dendrites, and OD provides the spatial configuration of the neurite structures based on the composite pattern of intra- and extracellular diffusivity [29]. For WM NDI measures, all subjects' NDI data were registered to a common space based on nonlinear transformation and projected to the WM tract skeleton. Next the major WM tract ROIs were then applied to the skeletonized WM NDI maps to extract ROI-wise NDI measures [26]. For the GM diffusivity assessment, diffusion modeling parameters were determined by voxel wise iterative parameter selection method. We used the maximum likelihood estimation of model fitting error to define the optimal intrinsic free diffusivity parameters [30]. The optimal parameters were used to reconstruct the GM NDI maps and then merged into the 78 GM ROIs to extract ROI-wise NDI measures [30,31].

2.5. T1-Weighted MRI Measures

From the Freesurfer cortical reconstruction process of T1W-MRI, we extracted six measures per subject, including cortical thickness, cortical surface area, cortical volume (cVolume), subcortical GM volume (scVolume), WM volume, curvature (curv). Specifically, cortical thickness, surface area, volume, and curvature are extracted from 62 ROIs based on Desikan–Killiany–Tourville (DKT) atlas, while subcortical ROIs are defined by Freesurfer built-in atlas [31,32].

2.6. Statistical Analysis

From the data processing steps, we generated in total 14 types of imaging measures: 4 NDI, 4 DTI, and 6 T1-weighted morphometric measures. For each type of imaging measure, we conducted statistical comparisons of GWI cases vs. controls using linear regression models adjusting for age and sex, and then corrected for multiple comparison using false discovery rate (FDR) [33]. We reported t-values and FDR-corrected p-values (FDR-p), significant features are defined as FDR-$p < 0.05$.

2.7. Machine Learning Classification

Imaging measures described in the previous sections are used as pre-defined features for training ML classification models. Age- and sex-related confounds were removed from the raw data before training the model. This step is achieved by estimating the effects of age and sex on imaging measures using a linear regression model that is similar to a method applied in an early study [19]. For building the classifier for each imaging measure we adapted a reinforcement learning algorithm with artificial bee colony algorithm for feature selection (BSO: bee swarm optimization), and the K nearest neighbors (KNN) algorithm for classification training and performance evaluation [34,35].

2.7.1. Feature Space Selection and Classifier Training

As mentioned previously, some specific neuroimaging markers (i.e., NDI measures) may be more sensitive for detecting the subtle neurological changes occurring in GWI cases [16]. For training the classifiers, each type of imaging measures (i.e., measurement domains) serves as prior information that will allow us to set up specific feature space for potentially better ML outcomes. Within each feature space, reinforcement learning-based BSO (QBSO) was used to perform iterative search of the subset of features that provides the best classification performance on the training dataset (more details described in QBSO Tuning). Through QBSO, a final subset of features (final solution) was selected to build a final classifier. Final classifiers trained on each feature space were- then tested on the validation dataset (see more details in Ensemble Approach).

QBSO Tuning

This feature selection concept combines the BSO and reinforcement learning (specifically Q-learning) to upgrade simple local search to a more adaptive and efficient search for the final

solution [34,35]. Previous study has shown that this hybrid method outperforms other well-known ML algorithms for feature selection [35]. More specifically, the BSO method mimics the foraging behavior of natural bees by performing iterative local search for an optimized solution [36].

From the predefined feature space explained earlier, the initial solution is randomly generated. Then, BSO randomly modifies the initial solution to multiple different secondary solutions, where each will be assigned to a bee (an agent) to perform local search to find local optimum (based on k-fold cross-validation accuracy). In this local search stage, each bee refers to a series of experiments obtained in previous steps to make a decision to do further search in the current search pace, and this local search will continue until no further improvement of accuracy occurs. When the bee reaches this point, each bee's search history is shared to other bees and used for the diversification of searching process.

In the diversification process, the most distant solution will be selected based on the shared information. During this process, the role of reinforcement learning is to allow the agent learn through an interactive environment by trial and error. As the result, the QBSO method will search for a solution (i.e., resulting feature list) that maximizes the reward through multiple iterations. In each iteration, KNN runs on the candidate features (one of the secondary solutions) selected from the bee and tested for 5 iterations of 5-fold cross-validation on the training dataset. We used an average accuracy measure from the 5-fold cross-validation for estimating the reward. Finally, the search process will terminate based on the pre-defined parameters. To set up the optimal parameters, we used a grid-search strategy that is empirically searching the parameters resulting in the highest classification accuracy for the training dataset. The final parameters used in this experiment are listed as follows: flip: 20, max. chance: 9, nBees: 30.

Ensemble Approach

Per each feature space (i.e., one type of imaging measure), QBSO produces a subset of final features that provides the highest average accuracy from the iterative search. QBSO is repeated 5 times in total to generate 5 final solution candidates for a single training dataset. Per each solution, we built 3 different classifiers- KNN, support vector machine, and random forest classifiers. The training dataset was further split into 2 parts (i.e., training and testing) and used to train each classifier. Then the weighted majority voting was used to ensemble those 15 classifiers (i.e., 3 classifiers from each solution) to make a final prediction on the validation dataset. The following weight function was used: $Wi = Pi/(1 - Pi)$, Pi: performance of i-th classifier, i = [1:15].

2.7.2. Comparing Classification with Different Imaging Measures

As mentioned previously, each type of imaging measures was used to set up distinct candidate feature space for training the classifiers. The resulting 14 different classifiers (4 NDI, 4 DTI, and 6 T1W-MRI morphometric measures) were evaluated based on their classification performances. For the benchmark testing, the entire dataset was initially divided into a training dataset and a validation dataset based on a 5-fold partitioning. We took one fold as a validation dataset and used the remaining 4-fold data for performing the QBSO training framework (Section 2.7.1). This process was repeated 5 times as training/validation datasets rotate among the 5 folds (by taking each fold as the validation dataset in each iteration). For the classification performance comparison, we reported performance measures (averaged from 5 iterations after validation) of accuracy, sensitivity, specificity, and F-score. We included F-score as a more representative performance measure for the imbalanced case and control groups [37]. In addition to the average accuracy, we included the standard deviation (SD) of accuracy, as an estimate of variations between iterations, and the highest accuracy value for the top three classifiers.

3. Results

3.1. Group-Level Statistical Comparison and Key Imaging Features

Statistical analysis of NDI measures showed significant differences between GWI cases and controls in both WM tracts and GM ROIs (FDR-$p < 0.05$) (Figure 1). The full result can be found in Table S1. All major WM tracts showed significant decreases in ND and OD for GWI cases compared to controls (Figure 1A). The greatest significant group differences between GWI cases and controls were seen in the bilateral corticospinal tract (CST, t = −3.119 FDR-$p = 0.017$ (left), t = −3.129, FDR-$p = 0.017$ (right)) and the bilateral anterior thalamic radiations (ATR, t = −2.891, FDR-$p = 0.017$ (left), t = −2.808, FDR-$p = 0.017$ (right)) for WM ND, and in the bilateral cingulum cingulate gyrus bundle (CCG, t = −4.041 FDR-$p = 0.002$ (left), t = −3.384, FDR-$p = 0.007$) for WM OD. Both ND and OD showed decreased patterns (FDR-$p < 0.05$) for most GM ROIs as well (Figure 1B). The greatest significant group differences between GWI cases and controls were seen in the left isthmus of cingulate gyrus (t = −3.319, FDR-$p = 0.036$) and the bilateral thalamus proper (t = −3.168, FDR-$p = 0.036$ (left), t = −3.015, FDR-$p = 0.036$) for GM ND, and in the bilateral caudal anterior cingulate gyrus (t = −3.262, FDR-$p = 0.016$(left), t = −3.182, FDR-$p = 0.016$ (right)), the bilateral posterior cingulate gyrus (t = −3.832, FDR-$p = 0.016$ (left), t = −2.461, FDR-$p = 0.03$ (right)), the bilateral amygdala (t = −3.593, FDR-$p = 0.016$ (left), t = −3.516, FDR-$p = 0.016$ (right)) and the bilateral putamen (t = −3.228, FDR-$p = 0.016$ (left), t = −3.134, FDR-$p = 0.016$ (right)) for GM OD. The full list of statistically significant imaging features can be found in Table S1.

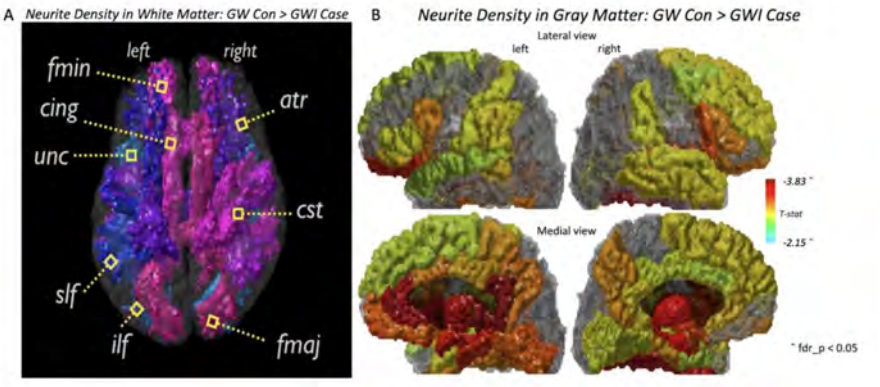


Figure 1. Gulf War illness (GWI) cases vs. Gulf War (GW) control group comparisons of gray matter (GM) and white matter (WM) neurite density imaging (NDI) measures and summary of significant regions. (**A**) 3D tract representation of significant WM ND differences between GWI case and control groups. (**B**) 3D region of interest (ROI) representation of significant GM ND differences between GWI case and control groups. Color bar corresponds to the magnitude of t-value, red indicates greater difference between groups, and vice versa. Fmaj = corpus callosum forceps major, Fmin = corpus callosum forceps minor, atr = anterior thalamic radiations, cst = corticospinal tract, cing = cingulum cingulate gyrus bundle, ilf = inferior longitudinal fasciculus, slf = superior longitudinal fasciculus, unc = uncinate fasciculus.

3.2. Machine Learning Classification Performance

As shown in Figure 2 and Table 2, the best classifier for GWI cases vs. control we had is trained using the WM OD measures, which achieved F-score of 0.941, an accuracy of 90% (SD: 0.063, highest accuracy: 91.7%), sensitivity of 95%, and specificity of 65%. The specific features include the left CST, the corpus callosum forceps minor (fminor), the left inferior fronto-occipital fasciculus (IFOF), the left inferior longitudinal fasciculus (ILF), the left superior longitudinal fasciculus (SLF), and the left superior longitudinal fasciculus temporal (SLFT). All features were statistically significant based on group-level analysis (Figure 1A, Table S1). The second-best classifier is trained using the GM ND measures, which achieved F-score of 0.922, an accuracy of 86.7% (SD: 0.054, highest accuracy:

91.7%), sensitivity of 96%, and specificity of 40%. The specific features used by this GM ND classifier include both cortical and subcortical structures of the limbic system, including the bilateral caudal anterior cingulate gyri (Table 2). The third best classifier was trained using the WM ND measures, which achieved F-score of 0.914, an accuracy of 85% (SD: 0.048, highest accuracy: 91.7%), sensitivity of 96%, and specificity of 30%. For this classifier, the specific features included the bilateral anterior thalamic radiations (ATR), the bilateral IFOF, the bilateral ILF, the left SLF, the right SLFT and the Fminor (Table 2). The full list of imaging features used by the top three classifiers can be found in Table 2 and the full list of classifier performances can be found in Table S2.

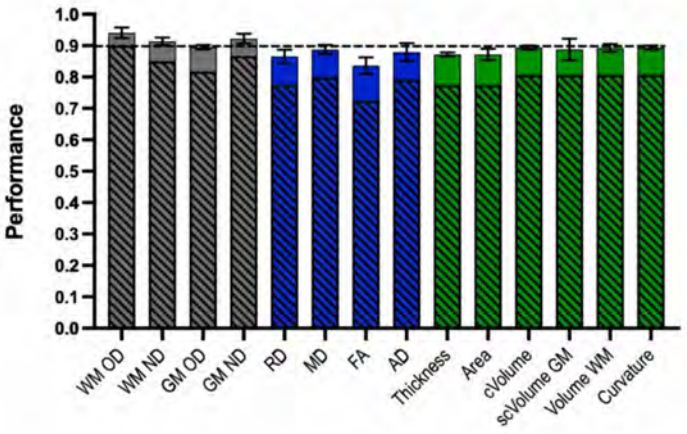


Figure 2. Classification performances of all classifiers. Each bar represents the performance (solid-colored bar: average F-score, shaded area: average accuracy) of each type of classifier trained on one imaging measure, data is presented as mean ± SEM after cross-validation. Grey-colored bars: NDI measure-based classifiers. Blue-colored bars: diffusion tension imaging (DTI) measure-based classifiers. Green-colored bars: T1-weighted structural MRI (T1W-MRI) measure-based classifiers. WM OD = white matter orientation dispersion, WM ND = white matter neurite density, GM OD = grey matter orientation dispersion, GM ND = grey matter neurite density, RD = radial diffusivity, MD = mean diffusivity, FA = fractional anisotropy, AD = axial diffusivity, thickness = cortical thickness, area = cortical surface area, cVolume = cortical volume, scVolume = subcortical GM volume, volume WM = white matter volume.

Table 2. Summary of classification performances and feature characteristics.

Measure	ACC	SEN	SPE	F-Score	Key Features	
WM OD	90%	95%	65%	0.941	L CST ** L IFOF ** L ILF ** L SLF ** L SLFT ** Fminor **	
GM ND	86.7%	96%	40%	0.922	L caudal anterior cingulate * L cuneus L inferior temporal L paracentral * L posterior cingulate * L thalamus proper *	R caudal anterior cingulat R lingual R pars orbitalis R amygdala * R putamen *
WM ND	85%	96%	30%	0.914	L ATR * L IFOF * L ILF * L SLF * Fminor *	R ATR * R IFOF * R ILF * R SLFT *

ACC: accuracy, SEN: sensitivity, SPE: specificity, F-score: F1 score, WM OD: white matter orientation dispersion index, GM ND: gray matter neurite density index, WM ND: white matter neurite density index, L: left hemisphere, R: right hemisphere, CST: corticospinal tract, IFOF: inferior fronto-occipital fasciculus, ILF: inferior longitudinal fasciculus, SLF: superior longitudinal fasciculus, SLFT: superior longitudinal fasciculus temporal, Fminor: corpus callosum forceps minor, ATR: anterior thalamic radiation. *: FDR-$p < 0.05$ in group-level statistical comparison. **: FDR-$p < 0.01$ in group-level statistical comparison.

4. Discussion

In this study, we used various neuroimaging techniques (NDI, DTI, structural T1W-MRI) to identify important features that may help to differentiate between veterans with GWI and control veterans. These features were selected through two different analytical frameworks: (1) group-level statistical analysis, and (2) single subject-level ML classification models. From our group-level, univariate analysis, we identified important imaging features, especially from WM and GM NDI and T1W-MRI regional volumetric measures, which showed high contrasts between veterans with GWI and control veterans. From the multivariate classification results, we could additionally identify unique imaging features that are important for making single-subject level inferences regardless of its relevance to the group differences.

The results from the group-level statistical analysis showed that NDI measures are the most sensitive marker for detecting GWI pathology than other types of neuroimaging measures. For WM NDI measures, all major tracts showed significant decreases for veterans with GWI compared to control veterans (Figure 1A). The greatest significant group differences were seen in the bilateral CST for WM ND and bilateral CCG bundle for WM OD (Table S1). The roles of these tracts in many essential physical and neuropsychological functions have been well described by previous literatures. For instance, earlier studies showed that disruption of the CST WM integrity was associated with motor impairment that occurs in the early stages of many neurological conditions such as Huntington's Disease and Multiple Sclerosis [38,39]. Similarly, disruption of CCG has been associated with impaired executive functioning, pain, memory deficits, and has been a main target for conditions including major depression, schizophrenia, post-traumatic stress disorder (PTSD), and autism spectrum disorder [40]. Changes in these tracts captured by our WM NDI results may also be important to understand specific symptoms such as muscle pain, fatigue, and depression observed in GWI.

From the ML framework, we confirmed that WM OD, GM ND, and WM ND measures were the sources of the top three classifiers (based on average accuracy) (Figure 2, Table 2). The classifier trained using the WM OD measure showed the best performance and consistently reporting six features: the left CST, IFOF, ILF, SLF, SLFT, and the Fminor (Table 2). Due to the completely imbalanced distribution of the data used in this study, performance on classifying controls were more challenging in QBSO and this calls better ideas on handling this issue. For example, synthetic oversampling method such as the synthetic minority oversampling technique (SMOTE) may help addressing this issue [41]. Additionally, in this type of imbalanced sample, assessing the F1-score might serve as a more realistic measure of the classification performance [37]. Although we used average accuracy measure for comparing classifiers, WM OD showed a high F-score (0.941), showing that our proposed ML framework is providing reasonable performance at least in this sample. Compared to the NDI classifiers, the classifiers from DTI measures or T1W-MRI measures all had lower classification performance than NDI measures (Table S2). These results suggest that (1) NDI measures are important imaging markers for defining GWI, and (2) the features defined from ML framework provides distinct information from the group-level statistics on describing GWI. While several features from the group-level statistics may present with overlapping patterns to ML classifiers, there are also unique features reported by ML classifiers but not captured in the group-level analysis framework.

Both our findings on group-level statistics and single subject-level classification model demonstrated the importance of NDI measures for defining GWI. Moreover, considering the other ML methods tested on mild or preclinical stage illness, such as mild cognitive impairment staying with ~78% accuracy levels, the classification performance obtained from NDI QBSO is impressive and brings more attention into the complex diffusion imaging measures for studying preclinical stage or mildly progressive illness [42]. In the current study, we not only identified widespread statistically significant NDI features through group-level analysis, but also demonstrated that WM OD measures trained a better classifier compared to other imaging measures. This is consistent with our previous studies on NDI showing that this technique is sensitive to microstructural and macrostructural brain alterations and useful for detecting neurological abnormalities in GW veterans [16]. Our result also

corroborated with our previous findings that showed a higher sensitivity for the novel NDI measures compared to the common DTI measures (e.g., FA, MD, etc.). As we suggested before, this might be due to the higher specificity of NDI for detecting changes in different tissue components [16]. We previously found that there is a strong correlation between alterations in GM ND measure and worse self-reported fatigue and sleep symptoms, and with upregulated levels of proinflammatory cytokines TNFRI and TNFRII [16]. However, based on our current findings, GM ND measures provided slightly lower classification performance than WM OD and ND measures in this study. In addition, while classifier trained on WM OD resulted in nearly identical final solutions across five iterations of validation, GM measures resulted in more variabilities in the selected feature solutions. This might be due to the differences in dimensional size between WM and GM feature space. GM measures have more numbers of features (more complexity in the feature space) to be searched out during the QBSO process than WM measures, and thereby requiring more delicate optimization process especially in this not-a-large dataset problem. Although further investigations based on larger dataset is key to address the issue, this may also indicate that WM OD measures can be better markers for simply classifying veterans with GWI from control veterans, while GM ND can be a sensitive marker to specific symptom domains. Our results also support the diagnostic value of these NDI markers for clinical applications.

Altogether, these results suggest that the microstructural changes measured by NDI may be attributed to GM and WM deficits following chronic neuroinflammation. In line with this finding, other studies have shown that chronic neuroinflammation related to GWI symptoms may be a result of both morphological and functional changes that occurred in glial cells. For instance, a study using a rat model of GWI showed that exposure to the chemical agent, diisopropyl fluorophosphate (DFP: a sarin surrogate), was associated with fewer numbers of both mature and dividing oligodendrocytes in the prefrontal cortex, which in turn interrupted the neuron-glial interactions [43]. DFP injection also induced neuroinflammation and neurodegeneration in multiple brain regions, which is associated with impaired contextual fear learning in these rats [44]. Similarly, mice exposed to DFP demonstrated epigenetic changes to genes related to the immune and neuronal systems and altered proportions of myelinating oligodendrocytes in the frontal cortex, which led to disrupted synaptic connectivity and WM alterations in GWI [45]. A recent in-vivo positron emission tomography study corroborated these findings and reported elevated levels of translocator protein (TSPO), a protein upregulated in activated microglia and astrocytes, in veterans with GWI compared to control veterans [46]. This elevation pattern was observed in many areas including the precuneus, prefrontal, primary motor, and somatosensory cortices [46]. Considering this evidence, our current findings further support the importance of novel NDI measures for detecting microstructural changes in the brain following chronic neuroinflammation in GWI.

Besides NDI measures, some T1W-MRI measures also demonstrated good performances for classifying veterans with GWI vs. control veterans. Among classifiers trained using T1W-MRI measures, the cortical volume, subcortical volume, WM volume, and mean curvature models achieved 80.8% accuracy, and highlighted key features in the frontal and temporal regions (Table S2). The results on the group-level statistical analysis also showed reduced volumes of frontal regions among veterans with GWI (Table S1). GM atrophy has been well studied as a hallmark for various neuropsychological disorders. Previous studies showed that reduced total cortical and regional frontal lobe volumes are associated with poor subjective sleep quality and increased self-reported frequency of hearing chemical alarm among GW veterans [12,47].

For DTI measures, the best performance was demonstrated by the MD classifier with an accuracy of 80% and F-score of 0.887 (Table S2). There is evidence that DTI measures may correlate with GWI symptom severity. An early study on GWI veterans showed that fatigue, pain, and hyperalgesia are associated with increased AD in the right IFOF [15]. Another study showed that changes in frontal-limbic WM connectivity, as indicated by reduced MD and increased FA in the right cingulate bundle, was associated with higher PTSD symptom severity score among a sample of 20 GW veterans [48]. In addition, GW veterans who had been exposed to chemical agents have increased

AD throughout many regions of the brain including the temporal stem, cingulum bundle, IFOF, etc., compared to unexposed veterans [13]. Through our results, we found that while T1W-MRI and DTI measures are less significant based on group-level statistical analysis, a subset of the regional measures may still explain key components of GWI symptoms.

In this study, we showed that neuroimaging markers help to identify GWI Nevertheless, we are expecting that the current approach can be improved in several aspects. One of the limitations of the current work is the imbalanced sample size, where the number of case subjects greatly exceeded the control subjects for building the classification model. This issue is reflected by the higher sensitivity and lower specificity for all the classifiers. To better handle this issue, we are planning to employ an oversampling method on the minority group to balance the samples. In our follow up work, we will also expand our analysis to a larger GW cohort including more control veterans recruited from other sites. Another important future direction is to test if the combination of multiple imaging measures, or combination of imaging and clinical measures (e.g., cognitive scores, inflammatory profiles, etc.) can improve the classification performance. This multivariate approach will be useful for identifying important features from large datasets. In conclusion, our current work provided the first evidence that novel NDI measures are not only useful for defining GWI based on the conventional group-level statistical comparisons, but also constitute key features for building single-subject level ML models for automated diagnostic classification. The features that are highlighted by our analysis suggest neurological changes underlying GWI pathology and support neuroinflammation as a potential target for therapeutic interventions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3425/10/11/884/s1, Table S1: List of key imaging features based on group-level statistical comparison, Table S2: The classification performance for all classifiers.

Author Contributions: Data analysis and paper writing is done by Y.G. and C.-H.C. W.C. and Y.Z. equally contributed to the ML software development. S.K. helped formal analysis. M.K., P.J., and R.T. worked on data curation. E.Y. and J.-H.K. helped ML optimization. R.B. helped formal analysis and validation. L.S. contributed conceptualization of the research. K.S. contributed conceptualization, funding acquisition, and project administration. B.-B.K. contributed idea development, study design, funding acquisition, leading the technical development, supervision of research works and writing. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by a Department of Defense CDMRP new investigator award (W81XWH-17-1-0440), CDMRP GWI consortium award (W81XWH-13-2-0072) and BBRAIN project (W81XWH-18-1-0549).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. White, R.F.; Steele, L.; O'Callaghan, J.P.; Sullivan, K.; Binns, J.H.; Golomb, B.A.; Bloom, F.E.; Bunker, J.A.; Crawford, F.; Graves, J.C.; et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: Effects of toxicant exposures during deployment. *Cortex* **2016**, *74*, 449–475. [CrossRef] [PubMed]
2. Janulewicz, P.; Krengel, M.; Quinn, E.; Heeren, T.; Toomey, R.; Killiany, R.; Zundel, C.; Ajama, J.; O'Callaghan, J.; Steele, L.; et al. The Multiple Hit Hypothesis for Gulf War Illness: Self-Reported Chemical/Biological Weapons Exposure and Mild Traumatic Brain Injury. *Brain Sci.* **2018**, *8*, 198. [CrossRef] [PubMed]
3. Yee, M.K.; Seichepine, D.R.; Janulewicz, P.A.; Sullivan, K.A.; Proctor, S.P.; Krengel, M.H. Self-Reported Traumatic Brain Injury, Health and Rate of Chronic Multisymptom Illness in Veterans from the 1990–1991 Gulf War. *J. Head Trauma Rehabil.* **2016**, *31*, 320–328. [CrossRef] [PubMed]
4. Yee, M.K.; Janulewicz, P.A.; Seichepine, D.R.; Sullivan, K.A.; Proctor, S.P.; Krengel, M.H. 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 Sci.* **2017**, *7*, 79. [CrossRef]
5. O'Callaghan, J.P.; Kelly, K.A.; Locker, A.R.; Miller, D.B.; Lasley, S.M. Corticosterone primes the neuroinflammatory response to DFP in mice: Potential animal model of Gulf War Illness. *J. Neurochem.* **2015**, *133*, 708–721. [CrossRef]

6. Gade, D.M.; Wenger, J.B. Combat exposure and mental health: The long-term effects among US Vietnam and Gulf War veterans. *Health Econ.* **2011**, *20*, 401–416. [CrossRef]
7. Rathbone, A.T.; Tharmaradinam, S.; Jiang, S.; Rathbone, M.P.; Kumbhare, D.A. A review of the neuro- and systemic inflammatory responses in post concussion symptoms: Introduction of the "post-inflammatory brain syndrome" PIBS. *Brain Behav. Immun.* **2015**, *46*, 1–16. [CrossRef]
8. Steele, L. 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.* **2000**, *152*, 992–1002. [CrossRef]
9. Dursa, E.; Barth, S.; Porter, B.; Schneiderman, A. Gulf War Illness in the 1991 Gulf war and Gulf era veteran population: An application of the centers for disease control and prevention and Kansas case definitions to historical data. *J. Mil. Veterans Health* **2018**, *26*, 43–50.
10. Proctor, S.P.; Heeren, T.; White, R.F.; Wolfe, J.; Borgos, M.S.; Davis, J.D.; Pepper, L.; Clapp, R.; Sutker, P.B.; Vasterling, J.J.; et al. Health status of Persian Gulf War veterans: Self-reported symptoms, environmental exposures and the effect of stress. *Int. J. Epidemiol.* **1998**, *27*, 1000–1010. [CrossRef]
11. Chao, L.L.; Abadjian, L.; Hlavin, J.; Meyerhoff, D.J.; Weiner, M.W. Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: A study at 4T. *Neurotoxicology* **2011**, *32*, 814–822. [CrossRef] [PubMed]
12. Chao, L.L.; Kriger, S.; Buckley, S.; Ng, P.; Mueller, S.G. Effects of low-level sarin and cyclosarin exposure on hippocampal subfields in Gulf War Veterans. *Neurotoxicology* **2014**, *44*, 263–269. [CrossRef] [PubMed]
13. Chao, L.L.; Zhang, Y.; Buckley, S. Effects of low-level sarin and cyclosarin exposure on white matter integrity in Gulf War Veterans. *Neurotoxicology* **2015**, *48*, 239–248. [CrossRef] [PubMed]
14. Chao, L.L.; Zhang, Y. Effects of low-level sarin and cyclosarin exposure on hippocampal microstructure in Gulf War Veterans. *Neurotoxicol. Teratol.* **2018**, *68*, 36–46. [CrossRef]
15. Rayhan, R.U.; Stevens, B.W.; Timbol, C.R.; Adewuyi, O.; Walitt, B.; VanMeter, J.W.; Baraniuk, J.N. Increased Brain White Matter Axial Diffusivity Associated with Fatigue, Pain and Hyperalgesia in Gulf War Illness. *PLoS ONE* **2013**, *8*, e58493. [CrossRef] [PubMed]
16. Cheng, C.H.; Koo, B.B.; Calderazzo, S.; Quinn, E.; Aenll, K.; Steele, L.; Klimas, N.; Krengel, M.; Janulewicz, P.; Toomey, R.; et al. Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. *Brain Behav. Immun.* **2020**, *89*, 281–290. [CrossRef]
17. Mateos-Pérez, J.M.; Dadar, M.; Lacalle-Aurioles, M.; Iturria-Medina, Y.; Zeighami, Y.; Evans, A.C. Structural neuroimaging as clinical predictor: A review of machine learning applications. *Neuroimage Clin.* **2018**, *20*, 506–522. [CrossRef]
18. Sakai, K.; Yamada, K. Machine learning studies on major brain diseases: 5-year trends of 2014–2018. *Jpn. J. Radiol.* **2019**, *37*, 34–72. [CrossRef]
19. Moradi, E.; Pepe, A.; Gaser, C.; Huttunen, H.; Tohka, J. Alzheimer's Disease Neuroimaging Initiative. Machine learning framework for early MRI-based Alzheimer's conversion prediction in MCI subjects. *Neuroimage* **2015**, *104*, 398–412. [CrossRef]
20. Provenzano, D.; Washington, S.D.; Rao, Y.J.; Loew, M.; Baraniuk, J. Machine Learning Detects Pattern of Differences in Functional Magnetic Resonance Imaging (fMRI) Data between Chronic Fatigue Syndrome (CFS) and Gulf War Illness (GWI). *Brain Sci.* **2020**, *10*, 456. [CrossRef]
21. Ngiam, K.Y.; Khor, I.W. Big data and machine learning algorithms for health-care delivery. *Lancet Oncol.* **2019**, *20*, e262–e273. [CrossRef]
22. Buysse, D.J.; Reynolds, C.F., 3rd; Monk, T.H.; Berman, S.R.; Kupfer, D.J. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Res.* **1989**, *28*, 193–213. [CrossRef]
23. Melzack, R. The McGill Pain Questionnaire: Major properties and scoring methods. *Pain* **1975**, *1*, 277–299. [CrossRef]
24. Smets, E.M.; Garssen, B.; Bonke, B.; De Haes, J.C. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J. Psychosom. Res.* **1995**, *39*, 315–325. [CrossRef]
25. Fischl, B. FreeSurfer. *Neuroimage* **2012**, *62*, 774–781. [CrossRef]
26. Smith, S.M.; Jenkinson, M.; Johansen-Berg, H.; Rueckert, D.; Nichols, T.E.; Mackay, C.E.; Watkins, K.E.; Ciccarelli, O.; Cader, M.Z.; Matthews, P.M.; et al. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *Neuroimage* **2006**, *31*, 1487–1505. [CrossRef]

27. Wakana, S.; Jiang, H.; Nagae-Poetscher, L.M.; van Zijl, P.C.; Mori, S. Fiber tract-based atlas of human white matter anatomy. *Radiology* **2004**, *230*, 77–87. [CrossRef]
28. Mori, S.; Wakana, S.; Van Zijl, P.C.; Nagae-Poetscher, L.M. *MRI Atlas of Human White Matter*; Elsevier: New York, NY, USA, 2005.
29. Zhang, Z.; Schneider, T.; Wheeler-Kingshott, C.; Alexander, D. NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage* **2012**, *61*, 1000–1016. [CrossRef]
30. Fukutomi, H.; Glasser, M.F.; Zhang, H.; Autio, J.A.; Coalson, T.S.; Okada, T.; Togashi, K.; Van Essen, D.C.; Hayashi, T. Neurite imaging reveals microstructural variations in human cerebral cortical gray matter. *Neuroimage* **2018**, *182*, 488–499. [CrossRef]
31. Desikan, R.S.; Ségonne, F.; Fischl, B.; Quinn, B.T.; Dickerson, B.C.; Blacker, D.; Buckner, R.L.; Dale, A.M.; Maguire, R.P.; Hyman, B.T.; et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* **2006**, *31*, 968–980. [CrossRef]
32. Fischl, B.; Dale, A.M. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 11050–11055. [CrossRef] [PubMed]
33. Noble, W.S. How does multiple testing correction work? *Nat. Biotechnol.* **2009**, *27*, 1135–1137. [CrossRef] [PubMed]
34. Sutton, R.S.; Barto, A.G. *Introduction to Reinforcement Learning*; MIT Press: Cambridge, MA, USA, 1998.
35. Sadeg, S.; Hamdad, L.; Remache, A.R.; Karech, M.N.; Benatchba, K.; Habbas, Z. QBSO-FS: A Reinforcement Learning Based Bee Swarm Optimization Metaheuristic for Feature Selection. In *Advances in Computational Intelligence*; Rojas, I., Joya, G., Catala, A., Eds.; IWANN 2019; Lecture Notes in Computer Science; Springer: Cham, Switzerland, 2019; Volume 11507, pp. 785–796. [CrossRef]
36. Karaboga, D.; Basturk, B. A powerful and efficient algorithm for numerical function optimization: Artificial bee colony (ABC) algorithm. *J. Glob. Optim.* **2007**, *39*, 459–471. [CrossRef]
37. Jeni, L.A.; Cohn, J.F.; De La Torre, F. Facing Imbalanced Data Recommendations for the Use of Performance Metrics. In Proceedings of the 2013 Humaine Association Conference on Affective Computing and Intelligent Interaction, Geneva, Switzerland, 2–5 September 2013; pp. 245–251. [CrossRef]
38. Phillips, O.; Squitieri, F.; Sanchez-Castaneda, C.; Elifani, F.; Griguoli, A.; Maglione, V.; Caltagirone, C.; Sabatini, U.; Di Paola, M. The Corticospinal Tract in Huntington's Disease. *Cereb. Cortex* **2015**, *25*, 2670–2682. [CrossRef] [PubMed]
39. Pawlitzki, M.; Neumann, J.; Kaufmann, J.; Heidel, J.; Stadler, E.; Sweeney-Reed, C.; Sailer, M.; Schreiber, S. Loss of corticospinal tract integrity in early MS disease stages. *Neurol. Neuroimmunol. Neuroinflamm.* **2017**, *4*, e399. [CrossRef] [PubMed]
40. Bubb, E.J.; Metzler-Baddeley, C.; Aggleton, J.P. The cingulum bundle: Anatomy, function, and dysfunction. *Neurosci. Biobehav. Rev.* **2018**, *92*, 104–127. [CrossRef]
41. Fernández, A.; García, S.; Herrera, F.; Chawla, N.V. SMOTE for learning from imbalanced data: Progress and challenges, marking the 15-year anniversary. *J. Artif. Int. Res.* **2018**, *61*, 863–905. [CrossRef]
42. Forouzannezhad, P.; Abbaspour, A.; Li, C.; Fang, C.; Williams, U.; Cabrerizo, M.; Barreto, A.; Andrian, J.; Rishe, N.; Curiel, R.E.; et al. A gaussian-based model for early detection of mild cognitive impairment using multimodal neuroimaging. *J. Neurosci. Methods* **2020**, *333*, 108544. [CrossRef]
43. Belgrad, J.; Dutta, D.J.; Bromley-Coolidge, S.; Kelly, K.A.; Michalovicz, L.T.; Sullivan, K.A.; O'Callaghan, J.P.; Douglas Fields, R. Oligodendrocyte involvement in Gulf War Illness. *Glia* **2019**, *67*, 2107–2124. [CrossRef]
44. Flannery, B.M.; Bruun, D.A.; Rowland, D.J.; Banks, C.N.; Austin, A.T.; Kukis, D.L.; Li, Y.; Ford, B.D.; Tancredi, D.J.; Silverman, J.L.; et al. Persistent neuroinflammation and cognitive impairment in a rat model of acute diisopropylfluorophosphate intoxication. *J. Neuroinflamm.* **2016**, *13*, 267. [CrossRef]
45. Ashbrook, D.G.; Hing, B.; Michalovicz, L.T.; Kelly, K.A.; Miller, J.V.; de Vega, W.C.; Miller, D.B.; Broderick, G.; O'Callaghan, J.P.; McGowan, P.O. Epigenetic impacts of stress priming of the neuroinflammatory response to sarin surrogate in mice: A model of Gulf War illness. *J. Neuroinflamm.* **2018**, *15*, 86. [CrossRef] [PubMed]
46. Alshelh, Z.; Albrecht, D.S.; Bergan, C.; Akeju, O.; Clauw, D.J.; Conboy, L.; Edwards, R.R.; Kim, M.; Lee, Y.C.; Protsenko, E.; et al. In-vivo imaging of neuroinflammation in veterans with Gulf War illness. *Brain Behav. Immun.* **2020**, *87*, 498–507. [CrossRef] [PubMed]

47. Chao, L.L.; Reeb, R.; Esparza, I.L.; Abadjian, L.R. Associations between the self-reported frequency of hearing chemical alarms in theater and regional brain volume in Gulf War Veterans. *Neurotoxicology* **2016**, *53*, 246–256. [CrossRef] [PubMed]
48. Bierer, L.M.; Ivanov, I.; Carpenter, D.M.; Wong, E.W.; Golier, J.A.; Tang, C.Y.; Yehuda, R. White matter abnormalities in Gulf War veterans with posttraumatic stress disorder: A pilot study. *Psychoneuroendocrinology* **2015**, *51*, 567–576. [CrossRef]

brain sciences

MDPI

Article

Brain–Immune Interactions as the Basis of Gulf War Illness: Clinical Assessment and Deployment Profile of 1990–1991 Gulf War Veterans in the Gulf War Illness Consortium (GWIC) Multisite Case-Control Study

Lea Steele [1,*], Nancy Klimas [2,3], Maxine Krengel [4], Emily Quinn [5], Rosemary Toomey [6], Deborah Little [7], Maria Abreu [2], Kristina Aenlle [2,3], Ronald Killiany [8], Bang-Bon Koo [8], Patricia Janulewicz [8], Timothy Heeren [5], Allison N. Clark [9], Joy Ajama [8], Joanna Cirillo [8], Gerardo Buentello [1], Vanesa Lerma [1], Janet K. Coller [10] and Kimberly Sullivan [8,*]

1 Veterans Health Research Program, Beth K. and Stuart C. Yudofsky Division of Neuropsychiatry, Department of Psychiatry and Behavioral Sciences, Baylor College of Medicine, Houston, TX 77030, USA; buentellojerry@prahs.com (G.B.); vanesa.lerma@bcm.edu (V.L.)
2 Institute for Neuroimmune Medicine, Dr. Kiran C. Patel College of Osteopathic Medicine, Nova Southeastern University, Fort Lauderdale, FL 33314, USA; nklimas@nova.edu (N.K.); mabreu1@nova.edu (M.A.); kaenlle@nova.edu (K.A.)
3 Department of Veterans Affairs, Miami VA Healthcare System, Miami, FL 33125, USA
4 Department of Neurology, Boston University School of Medicine, Boston, MA 02118, USA; mhk@bu.edu
5 Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA; eq@bu.edu (E.Q.); tch@bu.edu (T.H.)
6 Department of Psychological and Brain Sciences, Boston University College of Arts and Sciences, Boston, MA 02215, USA; toomey@bu.edu
7 Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center at Houston, Houston, TX 77030, USA; Deborah.M.Little@uth.tmc.edu
8 Department of Environmental Health, Boston University School of Public Health, Boston, MA 02118, USA; killiany@bu.edu (R.K.); bbkoo@bu.edu (B.-B.K.); paj@bu.edu (P.J.); ajamajoy@bu.edu (J.A.); jcirillo@bu.edu (J.C.)
9 H. Ben Taub Department of Physical Medicine and Rehabilitation, Baylor College of Medicine, Houston, TX 77030, USA; anclark@bcm.edu
10 Discipline of Pharmacology, School of Biomedicine, University of Adelaide, Adelaide, SA 5005, Australia; janet.coller@adelaide.edu.au
* Correspondence: Lea.Steele@bcm.edu (L.S.); tty@bu.edu (K.S.)

check for updates

Citation: Steele, L.; Klimas, N.; Krengel, M.; Quinn, E.; Toomey, R.; Little, D.; Abreu, M.; Aenlle, K.; Killiany, R.; Koo, B.-B.; et al. Brain–Immune Interactions as the Basis of Gulf War Illness: Clinical Assessment and Deployment Profile of 1990–1991 Gulf War Veterans in the Gulf War Illness Consortium (GWIC) Multisite Case-Control Study. *Brain Sci.* **2021**, *11*, 1132. https://doi.org/10.3390/brainsci11091132

Academic Editor: Isabelle M. Rosso

Received: 19 July 2021
Accepted: 24 August 2021
Published: 26 August 2021

Abstract: The Boston University-based Gulf War Illness Consortium (GWIC) is a multidisciplinary initiative developed to provide detailed understanding of brain and immune alterations that underlie Gulf War illness (GWI), the persistent multisymptom disorder associated with military service in the 1990–1991 Gulf War. The core GWIC case-control clinical study conducted in-depth brain and immune evaluation of 269 Gulf War veterans (223 GWI cases, 46 controls) at three U.S. sites that included clinical assessments, brain imaging, neuropsychological testing, and analyses of a broad range of immune and immunogenetic parameters. GWI cases were similar to controls on most demographic, military, and deployment characteristics although on average were two years younger, with a higher proportion of enlisted personnel vs. officers. Results of physical evaluation and routine clinical lab tests were largely normal, with few differences between GWI cases and healthy controls. However, veterans with GWI scored significantly worse than controls on standardized assessments of general health, pain, fatigue, and sleep quality and had higher rates of diagnosed conditions that included hypertension, respiratory and sinus conditions, gastrointestinal conditions, and current or lifetime depression and post-traumatic stress disorder. Among multiple deployment experiences/exposures reported by veterans, multivariable logistic regression identified just two significant GWI risk factors: extended use of skin pesticides in theater (adjusted OR = 3.25, $p = 0.005$) and experiencing mild traumatic brain injury during deployment (OR = 7.39, $p = 0.009$). Gulf War experiences associated with intense stress or trauma (e.g., participation in ground combat) were not associated with GWI. Data and samples from the GWIC project are now stored in a repository for use

by GWI researchers. Future reports will present detailed findings on brain structure and function, immune function, and association of neuroimmune measures with characteristics of GWI and Gulf War service.

Keywords: Gulf War illness; brain–immune interactions; military exposures; pesticides; traumatic brain injury; case-control study

1. Introduction

The 1990–1991 Persian Gulf War was among the most impressive military campaigns of the modern era. In response to Iraq's military invasion of neighboring Kuwait in August 1990, U.S. and Coalition forces flooded into the region over a period of months. The active combat offensive, U.S. codenamed Operation Desert Storm, began with air strikes in January 1991 and ended with a ceasefire in February 1991 after just four days of ground combat [1]. But after the successful execution of the Gulf War, a substantial number of military personnel returned home with difficult health problems that were not explained by familiar medical or psychiatric diagnoses [2–4]. This condition, now known as Gulf War illness (GWI), remains a serious problem for Gulf War veterans 30 years after the war [5–7].

Relatively little was understood about the nature or causes of GWI in the early years after Desert Storm. As the years passed, a series of population studies identified a consistent profile of excess symptoms that affected up to one third of Gulf War veterans [8–11]. Multiple studies, using multivariable assessment methods, were also consistent in characterizing the most prominent GWI risk factors from among numerous stressors and potentially hazardous exposures that Gulf War troops encountered in theater [12–17]. In addition, clinical studies conducted in Gulf War veteran populations identified a series of neurological, immune and other pathobiological alterations that significantly distinguished GWI cases from healthy controls [18–25]. In parallel, studies using animal models to simulate the exposure experiences of Gulf War military personnel identified chronic and/or delayed neurological, inflammatory, and behavioral changes that were consistent with veterans' chronic symptoms [26–30]. Taken together, preclinical, clinical, and population studies converged to indicate that the complex etiology and pathobiology of GWI involved persistent brain and inflammatory alterations likely triggered by a limited number of deployment exposures during the 1990–1991 Gulf War.

While these findings represented important progress for understanding GWI, there remained an urgent need for improved diagnosis and effective treatments for veterans who continued to suffer from this condition many years after the war. To address these objectives, the Office of Congressionally Directed Medical Research Programs (CDMRP) of the U.S. Department of Defense sponsored research consortia that enlisted scientific expertise in diverse disciplines to advance understanding, diagnosis and treatment of GWI. The Brain–Immune Interactions as the Basis of GWI: Gulf War Illness Consortium (GWIC) was developed as a multisite, interdisciplinary research program capable of integrating and building on GWI findings in multiple fields.

Headquartered at Boston University, the GWIC included multiple sites and coordinated projects to determine the specific neurological, inflammatory, and neuroimmune processes that underlie the symptoms of GWI, with the central objective of identifying GWI biomarkers and treatments. The ten GWIC participating institutions include five sites that conducted studies of veterans who served in the Gulf War (Boston University, Miami VA, Nova Southeastern University, Baylor College of Medicine, University of Adelaide) and five sites that conducted animal and in vitro GWI studies (U.S. Centers for Disease Control and Prevention (CDC), National Institutes of Health, University of Colorado, Drexel University, Temple University). A central feature of the GWIC has been coordination of clinical studies of Gulf War veterans with animal studies that characterize persistent effects of Gulf War exposures on the brain and on neuroimmune processes. Animal models are also used to

test therapeutic compounds that counteract these effects and have the potential to provide beneficial GWI treatments. Data and samples from the GWIC project are now stored in a repository for use by GWI researchers.

The present report provides an overview of the core GWIC clinical project, a large three-site GWI case-control study conducted in Boston, Miami, and Houston. This study provided multifaceted evaluation of brain and immune parameters in Gulf War veterans that included detailed assessments of immune function and immunogenetic factors, magnetic resonance imaging (MRI) assessment of brain structure and function, neuropsychological testing, and clinical assessment of general health and psychiatric status. Here we describe study methods and data collected for the three-site project, compare general health measures between GWI cases and controls, and identify deployment experiences and exposures found to be significantly associated with GWI case status. Future reports will provide in-depth results from GWIC brain imaging, brain function, and laboratory assessments to determine the specific neurological, cognitive, immune and genetic parameters that underlie the symptoms of GWI.

2. Materials and Methods

2.1. Study Recruitment, Screening, and Participation

Data were collected for the GWIC case-control study between 2015 and 2020 at three clinical study sites: Boston University, the Miami Department of Veterans Affairs Medical Center (VAMC), and Baylor College of Medicine in Houston. Project recruitment was conducted through extensive outreach efforts to inform Gulf War veterans about the study via veterans groups and meetings, media articles, social media, and veteran referrals. After initial contact with the research team to obtain study information, interested veterans were invited to participate in a structured telephone interview to determine their study eligibility. Consenting veterans answered questions about their Gulf War military deployment, medical history, and current health. Those who met eligibility criteria were provided additional study information and, if interested, were invited to participate in the full study, which required a 1-day study visit. At the study site, veterans received detailed study information and provided informed consent prior to participating in the series of research evaluations and testing included in the study protocol, as described below. Study protocol and informed consent documents were approved by institutional review boards at Boston University, Miami VAMC, and Baylor College of Medicine and reviewed by the U.S. Army Medical Research and Development Command's Office of Human Research Protections.

2.2. Eligibility Criteria and GWI Case Definition

Veterans were eligible for the study if they had deployed to the Gulf War Theater of Operations for any period between August 1990 and July 1991, were able to provide informed consent, and had not previously been diagnosed with any conditions designated as exclusionary for purposes of the GWIC project, as noted below. Primary GWI case/control status was determined using the Kansas GWI case definition criteria [10]. Additional data were collected to determine if veterans also met CDC criteria for chronic multisymptom illness (CMI), as defined by Fukuda et al. [8].

Briefly, the Kansas GWI case definition inclusionary criteria require that veterans endorse multiple and/or moderate to severe symptoms as problems that had persisted or recurred over six months in at least three of six defined symptom domains. Defined symptom domains include: (1) fatigue/sleep problems, (2) pain symptoms, (3) neurological/cognitive/mood symptoms, (4) gastrointestinal symptoms, (5) respiratory symptoms, and (6) skin symptoms. The Kansas criteria also exclude as GWI cases any veterans diagnosed with conditions that could account for their chronic symptoms or interfere with their ability to accurately report them (e.g., severe psychiatric disorders). Notably, Kansas GWI criteria do not exclude subjects with other unexplained symptom-defined conditions, such as fibromyalgia (FM), chronic fatigue syndrome/myalgic encephalomyelitis (CFS), or irritable bowel syndrome (IBS) [10].

Consistent with the Kansas GWI criteria, lead investigators at the three study sites (KS, NK, LS) established a list of medical and psychiatric conditions that were pre-specified as exclusionary for the GWIC study. The GWIC exclusionary criteria also designated time frame parameters to allow for prior conditions that had resolved or were adequately managed, and so could not account for veterans' symptoms at the time of the study.

Exclusionary conditions. The GWIC study eligibility criteria excluded veterans who had ever been diagnosed by a physician with multiple sclerosis, lupus, rheumatoid arthritis, stroke, Parkinson's disease, amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease), Alzheimer's disease, bipolar disorder, or schizophrenia. Criteria also excluded veterans if they had previously been diagnosed with any of the following conditions and there was any indication the condition was still active in the five years before the study: seizure disorder, heart disease (high blood pressure or high cholesterol not exclusionary), kidney disease, liver disease, cancer (except non-melanoma skin cancer, which was not exclusionary). For example, veterans who had a prior cancer diagnosis but had been told by their providers that they had been cancer–free for at least 5 years were not excluded from the study. Veterans diagnosed with diabetes were included only if their blood sugar had been well controlled for at least 2 years but were otherwise excluded. Veterans were also excluded if they had a chronic infectious disease lasting six months or longer or were recovering from a serious injury that could account for their symptoms. In addition, veterans were excluded if they had been hospitalized for post-traumatic stress disorder, depression, or alcohol or drug dependence in the previous 5 years. GWI exclusionary criteria established for the GWIC study are summarized in Supplemental Figure S1.

Exclusionary criteria were applied to all screened participants, regardless of their likely case status, to ensure a comparison group of healthy controls and minimize the potential for any case-control differences identified by the study to be the result of conditions other than GWI.

2.3. Data Collection

2.3.1. Screening Interview

Veterans who consented to the screening interview were asked if they had deployed to the Persian Gulf region for any period between August 1990 and July 1991, then responded to a series of questions about symptoms associated with Kansas GWI and CMI criteria. For symptoms identified as persistent problems over the prior 6 months, veterans were asked to rate the problem as mild, moderate or severe. Veterans were also asked about their medical history, including hospitalizations and conditions diagnosed by a healthcare provider that could potentially affect study eligibility. Eligible veterans were provided additional study information and invited to schedule an in-person study visit.

2.3.2. GWIC Study Visit

Upon arriving at the study site the morning of their appointment, veterans were given detailed study information, and any questions were discussed prior to obtaining informed consent. Consenting veterans provided fasting blood samples and initial saliva samples, followed by a brief physical evaluation to obtain data on height, weight, vital signs (including supine and standing blood pressure) and fibromyalgia tender points. Participants were then administered a neuropsychological testing battery that included tests of executive system functioning, attention, motor function, visuospatial function, memory, mood, and motivation. Veterans also received a clinical psychiatric interview that included the Clinician Administered PTSD Scale (CAPS) [31] and the Structured Clinical Interview for DSM-V (SCID) [32] to identify exclusionary diagnoses (bipolar disorder, schizophrenia) and/or comorbid psychiatric diagnoses (major depression, anxiety disorder, dysthymia, post-traumatic stress disorder (PTSD)). In addition, at the Boston and Houston sites, study participants with no safety contraindications received a magnetic resonance imaging (MRI) scan of the brain. The neuroimaging battery included a T1-weighted magnetization-prepared rapid acquisition (MPRAGE) sequence, multi-component T-2 scan,

diffusion tensor imaging (DTI), fMRI, pCASL sequence, and a High Angular Resolution Diffusion Imaging (HARDI DTI) scan [33,34].

2.3.3. Standardized Health Assessments and Study Questionnaires

During the course of their study visit, veterans were administered a series of standardized health assessments and completed additional questionnaires online during or after their study visit. Health assessments included the Veterans SF36 [35], McGill Pain Questionnaire [36], Pain Visual Analog Scale, Multidimensional Fatigue Inventory [37], Pittsburgh Sleep Quality Index [38], and the Structured Neurotoxicant Assessment Checklist (SNAC) [39]. Veterans also completed the Kansas Gulf War and Health Questionnaire [15], which queries a broad range of experiences and exposures specifically associated with Gulf War service and the duration of each exposure. In addition, veterans were asked if they had experienced one or more mild traumatic brain injuries (mTBI) before, during, or after Gulf War deployment, and the estimated number of mTBIs during each period. For purposes of the study, mTBI was defined according to American Academy of Neurology guidelines [40] as an impact to the head that causes symptoms for any amount of time (i.e., seconds or longer)—symptoms that may have included sensitivity to light or noise, headache, dizziness, balance problems, nausea, vomiting, trouble sleeping, fatigue, confusion, difficulty remembering, difficulty concentrating, or loss of consciousness.

2.3.4. Testing of Collected Samples

Blood analyses included a battery of standard clinical diagnostic tests (complete blood count, basic metabolic panel, thyroid panel, rheumatoid factor, antinuclear antibody). In addition, participants' de-identified blood and saliva samples were shipped by overnight courier to the project's central research laboratory and repository site, the E.M. Papper Immunology Laboratory at Nova Southeastern University, where extensive research testing was conducted. Research analyses included multiplex evaluation of a comprehensive panel of cytokines and chemokines in the blood and nanostring analyses of mRNA and miRNA of proteins associated with toll like receptor functioning and glial activation. In addition, cortisol levels were tested in saliva samples collected at regular intervals throughout the study visit. Saliva samples were also shipped to the GWIC collaborating genetic laboratory at the University of Adelaide to test for genetic markers associated with variability in immune and proinflammatory processes.

2.4. Data Management and Analyses

Data collected at the three clinical study sites, identified only by subjects' study identification numbers, were securely submitted to Boston University's Biostatistics and Epidemiology Data Analytics Center (BEDAC) for data consolidation, management, and analyses. Additional analyses were conducted by project investigators at individual study sites, to address specific research questions.

Data analyses for the current report involve bivariate and multivariable comparisons between GWI cases and controls using standard analytic methods. This included chi-square tests to evaluate case/control comparisons associated with categorical variables. Comparison of categorical outcomes for which any expected cell size was <5 utilized Fisher's exact test to determine *p*-values. Mean values of continuous variables were compared using T tests, according to observed distributions of individual variables. Significance was assessed by *p*-values determined using pooled variances, when equal for GWI cases and controls, and the Satterthwaite method [41,42] when variances were not equal. Prevalence odds ratios and 95% confidence intervals were used to estimate the magnitude of association of GWI with veteran-reported experiences/exposures during Gulf War deployment—both unadjusted (bivariate) associations and adjusted (multivariable) associations.

Multivariable logistic regression was used to identify independent associations of Gulf War experiences and exposures with GWI, adjusted for effects of covariates as well as

potential confounding effects of concurrent exposures. The multistep modeling approach first used results of bivariate analyses to test all significant associations of GWI with deployment experiences/exposures in a single model. Final models retained individual exposures significant at $p < 0.05$, as well as prominent variables (age, rank, PTSD) that differed between GWI cases and controls in initial bivariate analyses. All analyses were conducted using SAS/STAT statistical analysis software, version 9.4 [43].

3. Results

3.1. Study Sample

Overall, 703 veterans were screened for study eligibility at the three GWIC sites. Twenty-eight veterans indicated they had not served in theater for any period between August 1990 and July 1991 and were not further evaluated. Of the 675 remaining, 436 (65%) were identified as study eligible, and 239 were not eligible due to previous diagnoses of one or more exclusionary conditions. Four hundred eleven screened veterans were invited to participate in the full study, and 271 (66%) completed study appointments. Two veterans who completed study visits were subsequently excluded from the final sample based on additional health information obtained during study evaluations. The final GWIC study sample therefore included 269 Gulf War veterans: 223 GWI cases and 46 veteran controls. This included 147 veterans evaluated at the Boston site, 50 evaluated at the Miami site, and 72 evaluated at the Houston site.

Demographic, military, and deployment characteristics of the study sample are provided in Table 1. Overall, GWI cases were similar demographically to veteran controls but included a somewhat higher proportion of women (17% GWI cases vs. 9% controls, $p = 0.16$). Age group distributions were similar by decade, although the mean age of GWI cases was about two years younger than veteran controls ($p = 0.04$). Veterans' military characteristics at the time of the Gulf War were also similar, with one exception. The large majority of GWI cases (90%) had served in the enlisted ranks during the Gulf War, compared to only 70% of controls (OR =3.84, $p = 0.0003$). However, there were no case/control differences by military branch, service component, the time period veterans spent in theater, or the duration of veterans' deployment. Overall, 89% of all veterans in the sample had been in theater during the two months of active combat, January–February 1991. The remaining 11% either left the region during Operation Desert Shield, before the onset of air strikes, or arrived in theater after the cease-fire was declared in late February 1991.

Table 1. Demographic, military, and deployment characteristics of GWI cases and controls.

	GWI Cases (n = 223)	GW Veteran Controls (n = 46)	Test Statistic	p Value
Sex				
Female	17%	9%	2.01 [1]	$p = 0.16$
Male	83%	91%		
Age				
43–49	44%	30%	3.47 [1]	$p = 0.18$
50–59	45%	52%		
60+	11%	17%		
Mean age (years)	52.2	54.2	2.07 [2]	$p = 0.04$
Race				
Black/African American	13%	11%	0.18 [1]	$p = 0.92$
White/Caucasian	79%	80%		
Other/Mixed	8%	9%		
Hispanic ethnicity	9%	4%	na [3]	$p = 0.39$

Table 1. *Cont.*

	GWI Cases (n = 223)	GW Veteran Controls (n = 46)	Test Statistic	p Value
Highest Education Level			0.61 [1]	$p = 0.89$
High school or GED	6%	9%		
Some college or training after high school	49%	46%		
4 year degree	20%	20%		
Advanced degree	24%	26%		
Rank in 1990				
Enlisted	90%	70%	13.01 [1]	$p < 0.001$
Officer	10%	30%		
Branch of Service in 1990				
Army	65%	72%	2.47 [1]	$p = 0.48$
Navy	12%	15%		
Air Force	7%	4%		
Marines	16%	9%		
Service Component in 1990				
Regular (Active Component)	78%	76%	0.67 [1]	$p = 0.72$
Reserves	17%	15%		
National Guard	6%	9%		
Gulf War Deployment: Service Period in Theater				
Present Jan-Feb 1991 and departed by May 1991	71%	72%	0.91 [1]	$p = 0.63$
Present Jan-Feb 1991 and departed after May 1991	17%	21%		
Departed prior to Jan 1991 or arrived after February 1991	11%	7%		
Mean number of months in theater	6.5	6.7	0.45 [2]	$p = 0.65$

Abbreviations: GW = Gulf War; GWI = Gulf War illness; na = not applicable. Statistical tests: [1] chi-square; [2] T test; [3] Fisher's exact test.

3.2. General Health, Medical History, and Standardized Health Assessments

General health characteristics of GWI cases and controls are compared in Table 2. As shown, similar proportions of cases and controls recalled being in good to excellent health prior to Gulf War deployment, and were regular smokers both during the Gulf War and at the time of the study. As expected, however, GWI cases indicated worse overall health than controls at the time of the study. This disparity was reflected in significant case/control differences in veterans' medical history, and by standardized health and psychiatric assessments conducted at the time of the study.

Table 2. General health characteristics of GWI cases and controls.

	GWI Cases (n = 223)	GW Veteran Controls (n = 46)	Test Statistic	p Value
Veteran-reported health status prior to Gulf War deployment				
Excellent	92%	87%	1.24 [1]	$p = 0.26$
Good	8%	13%		
Veteran-reported health status at time of study				
Excellent	2%	20%	71.5 [1]	$p < 0.001$
Good	14%	50%		
Fair	37%	30%		
Poor	47%	0		

Table 2. *Cont.*

	GWI Cases (n = 223)	GW Veteran Controls (n = 46)	Test Statistic	p Value
Regular smoker				
During Gulf War deployment	27%	24%	0.24 [1]	$p = 0.62$
At time of study	10%	7%	na [3]	$p = 0.59$
Medical History: Physician-diagnosed conditions (not exclusionary for GWI)				
Hypertension	45%	28%	4.55 [1]	$p = 0.03$
Respiratory allergies/sinus problems	42%	7%	20.46 [1]	$p < 0.001$
Irritable bowel syndrome	31%	7%	11.62 [1]	$p < 0.001$
Other gastrointestinal diagnosis	31%	9%	9.35 [1]	$p < 0.01$
Chronic fatigue syndrome	23%	2%	10.71 [1]	$p < 0.001$
Asthma	14%	4%	3.02 [1]	$p = 0.08$
Chemical sensitivity	7%	0	na [3]	$p = 0.14$
Psychiatric Diagnoses: Evaluated at time of study				
Major Depression: Current or lifetime	44%	24%	5.54 [1]	$p = 0.02$
Dysthymia: Current or lifetime	7%	3%	1.00 [1]	$p = 0.32$
Anxiety Disorder: Current	14%	0	na [3]	$p = 0.01$
Post-traumatic Stress Disorder: Current or lifetime	56%	23%	15.43 [1]	$p < 0.001$
Standardized Health Assessments: Evaluated at time of study				
General Health				
Veterans SF-36 mean Physical Component Summary Score	35	50	12.86 [2]	$p < 0.001$
Veterans SF-36 mean Mental Component Summary Score	41	51	4.99 [2]	$p < 0.001$
Pain				
Magill Pain Inventory mean score (0–78)	32.5	16.4	−7.38 [2]	$p < 0.001$
Average pain level on best days (visual analog scale, 0–100)	26.6	10.1	−6.91 [2]	$p < 0.001$
Average pain level on worst days (visual analog scale, 0–100)	72.4	41.9	−7.38 [2]	$p < 0.001$
Fibromyalgia tender point exam				
Mean number of positive FM tender points (of 18)	6	1	−8.19 [2]	$p < 0.001$
Veterans with 11+ tender points	28%	2%	13.97 [1]	$p < 0.001$
Fatigue			−8.19 [2]	
Multidimensional Fatigue Inventory (MFI) mean score (0–100)	64.6	38.3		$p < 0.001$
Sleep				
Pittsburgh Sleep Quality Index mean score (0–21)	13.0	7.5	−9.12 [2]	$p < 0.001$
Physical evaluation at time of study				
Oral temperature (mean ^{o}F)	97.8	97.9	0.292 [2]	$p = 0.77$
Resting pulse (mean beats/minute)	70	68	−0.982 [2]	$p = 0.33$
Height (mean inches)	69	70	1.482 [2]	$p = 0.14$
Mean weight (pounds)	217	216	−0.292 [2]	$p = 0.77$
Body mass index (mean)	32	31	−1.002 [2]	$p = 0.32$
Supine blood pressure (mean systolic/mean diastolic)	134/82	134/79	-	ns
Standing blood pressure (mean systolic/mean diastolic)	132/88	131/86	-	ns
Diastolic drop of 10 or more points after standing	4%	2%	na [3]	$p = 1.00$
Systolic drop of 20 or more points after standing	6%	2%	na [3]	$p = 0.48$

Abbreviations: GW = Gulf War; GWI = Gulf War illness; FM = fibromyalgia; na = not applicable; ns = not statistically significant. Statistical tests: [1] chi-square; [2] T test; [3] Fisher's exact test.

As described, veterans who were previously diagnosed with any designated exclusionary conditions were not eligible for the study. As shown in Table 2, however, a significantly greater proportion of GWI cases than controls reported they had been diagnosed by a physician with a number of nonexclusionary medical conditions including hypertension, allergies or sinus problems, gastrointestinal conditions and two chronic multisymptom conditions: irritable bowel syndrome (IBS) and chronic fatigue syndrome (CFS). Structured psychiatric evaluations conducted for the study also indicated that a significantly greater proportion of GWI cases than controls met criteria for major depression (current or lifetime), anxiety disorder, and post-traumatic stress disorder (current or lifetime).

In addition, GWI cases scored significantly more poorly than controls on each of the standardized health assessments administered for the study. This included evaluations of general health and quality of life, pain, fatigue, and sleep quality. For example, whereas mean values for veteran controls on both the physical component (PCS) and mental component (MCS) summary scores of the Veterans SF36 were near the normal value of 50, GWI cases scored significantly worse on both the PCS (mean = 35, $p < 0.001$ vs. controls) and MCS (mean = 41, $p < 0.001$ vs. controls). Gulf War illness cases also reported significantly higher levels of pain on both their best and worst days and tested positive for a significantly greater number of fibromyalgia (FM) tender points than controls. Twenty-eight percent of GWI cases had 11 or more positive tender points out of 18 tested, consistent with 1990 diagnostic criteria for fibromyalgia [44], vs. only 2% of controls ($p < 0.001$).

Despite the significant degree of poor health indicated by both medical history and health assessments, veterans with GWI, overall, had mostly normal health indicators on standard physical evaluation and clinical diagnostic tests conducted for the study. As detailed in Table 2, no case/control differences were observed in relation to veterans' vital signs, height, weight, or body mass index. Few veterans exhibited possible evidence of postural orthostatic hypotension when comparing diastolic and systolic blood pressure moving from laying down to a standing position, with no significant differences between GWI cases and controls.

3.3. Blood Testing

Clinical reference lab testing of fasting blood samples taken the morning of the study identified only a limited number of differences between GWI cases and controls. Among basic metabolic panel (BMP) tests, GWI cases differed significantly from controls on mean levels of CO_2, glucose, and bilirubin. A greater proportion of GWI cases than controls had elevated fasting blood glucose levels (13% vs. 2%, $p = 0.04$), while a greater proportion of controls had higher-than-normal CO_2 and bilirubin levels. No significant case/control differences were associated with lipid panel tests, thyroid stimulating hormone, antinuclear antibodies, or rheumatoid factor. Of note, 38–42% of both cases and controls had elevated total and LDL serum cholesterol levels.

Complete blood count (CBC) testing also identified a limited number of case/control differences. These included significant differences in mean white blood cell (WBC) counts ($p = 0.007$), with more controls than cases having WBC counts below the reference range. Controls also had significantly greater mean percent monocytes than cases ($p = 0.04$), while cases had a larger mean red cell distribution width (RDW) than controls ($p = 0.03$).

3.4. Symptom Profiles: GWI Cases and Controls

Table 3 identifies the proportion of GWI cases and controls who endorsed each of the Kansas GWI criteria symptoms as persisting for six months or longer. Kansas GWI symptom criteria require two mild or one moderate-severe chronic symptom in at least 3 of 6 domains, a minimum of 3–6 symptoms. For the GWIC sample, however, each of the 29 individual GWI symptoms were endorsed by significantly more GWI cases than controls. Both GWI cases and controls endorsed symptoms at substantially higher frequencies than was typically observed in early Gulf War veteran studies. For example, over 90% of GWI cases endorsed fatigue, pain, and cognitive chronic symptoms, while 50% of controls

endorsed chronic joint pain and 41% had sleeping difficulties. Still, a significantly greater proportion of GWI cases than controls endorsed multiple or moderate-severe symptoms in each of the six defined symptom domains.

Table 3. Proportion of GWI cases and controls endorsing chronic symptoms.

Symptoms Identified as Persistent or Recurring Problems over the Previous 6 Months	GWI Cases [1] (*n* = 223)	GW Veteran Controls (*n* = 46)
Fatigue/Sleep Domain		
Not feeling rested after sleep	95%	35%
Fatigue	93%	22%
Problems getting to sleep or staying asleep	90%	41%
Feel unwell after physical exercise or exertion	79%	11%
Multiple or moderate-severe symptoms	99%	30%
Pain Domain		
Joint pain	94%	50%
Muscle pain	78%	11%
Body pain—hurt all over	70%	4%
Multiple or moderate-severe symptoms	92%	20%
Neurologic/Cognitive/Mood Domain		
Problems remembering recent information	91%	39%
Difficulty concentrating	90%	37%
Trouble finding words when speaking	83%	35%
Feeling irritable or having angry outbursts	80%	28%
Headaches	75%	13%
Feeling down or depressed	75%	33%
Numbness or tingling in extremities	71%	17%
Eyes sensitive to light	70%	20%
Feeling dizzy, lightheaded, or faint	65%	15%
Low tolerance for heat or cold	65%	9%
Night sweats	63%	11%
Symptomatic response to chemicals, odors	58%	9%
Blurred or double vision	55%	11%
Tremors or shaking	47%	4%
Multiple or moderate-severe symptoms	99%	57%
Gastrointestinal Domain		
Nausea or upset stomach	66%	4%
Diarrhea	64%	4%
Abdominal pain or cramping	61%	2%
Multiple or moderate-severe symptoms	72%	4%
Respiratory Domain		
Difficulty breathing or catching breath	63%	11%
Persistent cough when don't have a cold	53%	11%
Wheezing in chest	37%	4%
Multiple or moderate-severe symptoms	61%	2%
Skin Domain		
Skin rashes	52%	11%
Other skin problems	35%	2%
Multiple or moderate-severe symptoms	41%	2%
Mean number of GWI symptom domains for which multiple or moderate-severe symptoms were endorsed	4.7	1.1

Note: [1] Frequency of all individual symptoms and GWI symptom domains significantly greater in GWI cases vs. controls, $p < 0.001$. Abbreviations: GW = Gulf War; GWI = Gulf War illness.

We also assessed chronic symptoms associated with the CMI case criteria [8]. Nearly all GWI cases in our study (*n* = 221, 99%) met criteria for CMI, and half of veteran controls (*n* = 23, 50%) also met CMI criteria.

3.5. Association of Deployment Experiences and Exposures with GWI

Veterans reported a broad range of experiences and exposures during Gulf War deployment. Table 4 compares the overall proportion of GWI cases and controls who reported ever having each experience/exposure in theater, as well as the proportion who experienced each item for seven days or longer. Initial bivariate comparisons between GWI cases and controls suggested that 14 of the 23 Gulf War experiences/exposures queried were potentially associated with GWI, with unadjusted OR point estimates ranging from 1.99–8.33 ($p < 0.05$). A high degree of correlation was observed among reported deployment exposures, however, suggesting the potential for confounding error when evaluating exposure-GWI associations individually.

Table 4. Association of GWI case status with Gulf War deployment experiences and exposures.

Deployment Experiences/Exposures	% Exposed		OR (95% CI) (Unadjusted)	OR (95% CI) (Adjusted) [1]
	GWI Cases (n = 223)	GW Controls (n = 46)		
Regular smoker during deployment	27%	24%	1.20 (0.57–2.52)	1.14 (0.45–2.88)
Saw smoke from oil well fires				
Ever	87%	83%	1.41 (0.60–3.32)	0.74 (0.26–2.09)
≥7 days	66%	57%	1.47 (0.77–2.80)	0.92 (0.41–2.06)
Heard chemical alarms sounded				
Ever	86%	72%	2.43 (1.15–5.14) *	0.67 (0.25–1.80)
≥7 days	50%	28%	2.56 (1.28–5.14) *	1.36 (0.58–3.16)
Within 1 mile of exploding SCUD missile				
Ever	50%	33%	2.05 (1.05–4.01) *	1.24 (0.54–2.86)
≥7 days	15%	7%	2.60 (0.76–8.87)	2.10 (0.40–11.14)
Directly involved in ground combat				
Ever	45%	33%	1.70 (0.87–3.33)	0.69 (0.29–1.64)
≥7 days	21%	15%	1.47 (0.62–3.52)	0.44 (0.15–1.32)
Directly involved in air combat				
Ever	10%	7%	1.56 (0.44–5.47)	1.38 (0.30–6.38)
Saw U.S. troops badly wounded or killed				
Ever	54%	37%	2.00 (1.04–3.85) *	0.79 (0.34–1.81)
≥7 days	22%	13%	1.87 (0.75–4.67)	1.01 (0.33–3.10)
Saw Iraqis badly wounded or killed				
Ever	72%	57%	1.99 (1.03–3.82) *	0.73 (0.31–1.70)
≥7 days	33%	26%	1.37 (0.67–2.80)	0.80 (0.33–1.96)
Contact with prisoners of war				
Ever	59%	46%	1.69 (0.89–3.20)	0.99 (0.44–2.22)
≥7 days	32%	24%	1.47 (0.71–3.07)	1.29 (0.50–3.32)
Saw dead animals				
Ever	72%	59%	1.78 (0.92–3.43)	0.76 (0.31–1.83)
≥7 days	32%	26%	1.34 (0.65–2.74)	0.39 (0.15–1.06)
Saw destroyed enemy vehicles				
Ever	85%	72%	2.25 (1.07–4.74) *	0.95 (0.36–2.50)
≥7 days	57%	39%	2.04 (1.06–3.91) *	0.68 (0.29–1.60)
Contact with destroyed enemy vehicles				
Ever	71%	46%	2.87 (1.50–5.50) *	1.45 (0.61–3.43)
≥7 days	40%	24%	2.12 (1.02–4.40) *	0.91 (0.36–2.27)
Contact with American vehicles hit by friendly fire				
Ever	37%	28%	1.47 (0.73–2.97)	0.71 (0.28–1.82)
≥7 days	15%	17%	0.86 (0.37–2.01)	0.32 (0.10–1.03)

Table 4. *Cont.*

Deployment Experiences/Exposures	% Exposed		OR (95% CI) (Unadjusted)	OR (95% CI) (Adjusted) [1]
	GWI Cases (n = 223)	GW Controls (n = 46)		
Used pesticides cream/spray on skin				
Ever	74%	43%	3.69 (1.91–7.13) **	1.87 (0.87–4.02)
≥7 days	65%	26%	5.18 (2.53–10.59) **	3.25 (1.44–7.34) *
Wore uniform treated with pesticides				
Ever	53%	35%	2.16 (1.11–4.19) *	0.92 (0.36–2.40)
≥7 days	47%	24%	2.82 (1.36–5.84) *	1.19 (0.40–3.54)
Wore flea collars				
Ever	15%	4%	3.85 (0.89–16.67)	2.04 (0.38–10.86)
Saw living area sprayed/fogged with pesticides				
Ever	40%	24%	2.12 (1.02–4.40) *	0.91 (0.37–2.24)
≥7 days	23%	11%	2.48 (0.93–6.63)	1.17 (0.35–3.89)
Received one or more shots in arm in theater	75%	59%	2.52 (1.29–4.92) *	1.57 (0.70–3.51)
Received one or more shots in buttocks in theater	53%	37%	2.24 (1.16–4.32) *	1.23 (0.54–2.79)
Used pyridostigmine bromide (NAPP) pills				
Ever	82%	67%	2.25 (1.11–4.58) *	0.60 (0.23–1.56)
≥7 days	53%	37%	1.89 (0.98–3.64)	1.08 (0.49–2.38)
Contact with fresh CARC paint				
Ever	46%	13%	5.55 (2.25–13.65) **	2.13 (0.73–6.23)
≥7 days	27%	11%	2.96 (1.11–7.85) *	0.97 (0.30–3.09)
Experienced one or more mTBIs during deployment	37%	7%	8.33 (2.50–27.72) **	7.39 (1.64–33.28) *

Note: [1] Adjusted for use of skin pesticides ≥ 7 days, mTBI during deployment, age, rank, PTSD. * = significant association, $p < 0.05$; ** = significant association, $p < 0.001$. Abbreviations: GW = Gulf War; GWI = Gulf War illness, OR = odds ratio; CI = confidence interval; NAPP = nerve agent pyridostigmine pretreatment; CARC = chemical agent resistant coating; mTBI = mild traumatic brain injury; PTSD = posttraumatic stress disorder.

We therefore utilized logistic regression to identify independent associations of deployment experiences and exposures with GWI. After controlling for veterans' age, rank, PTSD status, and significant deployment experiences/exposures, adjusted models identified only two significant deployment risk factors for GWI. These included: (1) extended (≥7 days) use of cream or spray pesticides on the skin (adjusted OR = 3.25, $p = 0.005$) and (2) experiencing one of more mTBIs during deployment (adjusted OR = 7.39, $p = 0.009$).

In contrast, veterans who reported having one or more mTBIs prior to Gulf War deployment (38% cases vs. 35% controls, $p = 0.71$) or after the war (31% cases vs. 26% controls, $p = 0.50$) were not at increased risk for GWI. Further, no interactions were observed between deployment mTBI and pesticide use or other potential neurotoxicant exposures (e.g., hearing chemical alarms, use of pyridostigmine bromide) in relation to the risk for GWI.

Stressful deployment experiences were not identified as risk factors for GWI, although several were significantly associated with PTSD (not shown). For example, participation in ground combat was not a significant risk factor for GWI in our sample but was significantly associated with PTSD (OR = 3.55, $p < 0.001$).

4. Discussion

The GWIC case-control study, the core clinical project of the Boston University-based GWI research consortium, provided in-depth assessment of brain and immune function of 1990–1991 Gulf War veterans at three U.S. sites. Here we describe the general health of GWIC participants, results of clinical evaluation and testing of GWIC cases and controls, and significant GWI risk factors among veteran-reported wartime experiences and exposures.

In the three-site sample, GWI cases were generally similar to controls in relation to demographic, military, and deployment characteristics, although GWI cases were 2 years younger on average, and included a significantly higher proportion of veterans who had served in the enlisted ranks (vs. officers) during the war. This is similar to previous Gulf War veteran studies, where one of the most consistent findings has been a higher prevalence of GWI in enlisted personnel compared to officers [10,11,13,14]. This potentially reflects differences between officers and enlisted personnel in relation to Gulf War deployment activities and exposures. Such differences also parallel health differences observed in nonmilitary populations, for example, patterns of greater morbidity among civil servants serving in lower vs. higher ranks [45].

In addition to GWI symptoms, GWI cases had multiple indicators of poor health that distinguished them from controls. Veterans with GWI scored significantly worse on standardized assessments of general health status, pain, fatigue, and sleep quality compared to controls. They also reported a higher prevalence of physician-diagnosed hypertension, allergies and sinus problems, irritable bowel syndrome, other gastrointestinal disorders, and chronic fatigue syndrome, and were more likely to have current or lifetime PTSD and major depression.

Despite the degree of ill health associated with GWI, results of standard physical evaluation and clinical diagnostic tests were mostly normal, with only limited differences that distinguished GWI cases from healthy controls. This is consistent with earlier Gulf War veteran reports [8,46,47] and exemplifies a longstanding challenge associated with GWI for veterans and their healthcare providers. Symptoms, by definition, are patients' own experiences as opposed to externally "objective" measures of disease and have thus far been the only consistent marker of GWI in affected veterans. For many years, the lack of diagnosable abnormalities on physical exam and routine clinical lab tests commonly led to provider assumptions that nothing was wrong with veterans who reported chronic GWI symptoms, or that their health problems were the result of deployment stress and/or psychiatric in nature [48–50].

Such assumptions have not been supported by the large body of Gulf War population and clinical studies that have routinely indicated that GWI is not the result of serving in combat or other wartime stressors. Rather, the most consistently identified GWI risk factors have been neurotoxicant exposures during Gulf War deployment [11–17,51]. For the current study, GWIC participants reported a broad range of experiences and exposures during their wartime service. But only two—extended use of skin pesticides and having one or more mild traumatic brain injuries in theater—were significant risk factors for GWI. Our finding of extended personal pesticide use as a prominent GWI risk factor was consistent with previous studies [11–17,24]. The lack of association of GWI with serving in combat and other deployment stressors was also consistent with previous studies [11–15]. However, unlike previous studies, use of pyridostigmine bromide (nerve agent pyridostigmine pretreatment, or NAPP) pills was not identified as a risk factor for GWI in our study. The wide use of NAPP pills as a protective measure against potential deadly effects of chemical nerve agents was unique to the 1990–1991 Gulf War and was reported by a high proportion of both GWI cases (82%) and controls (67%) in our sample.

Having one or more mild TBIs during Gulf War deployment was also identified as a significant GWI risk factor in the current study, although mTBIs before or after the Gulf War were not. Brain injuries are commonly recognized as health concerns for veterans of post 9/11 deployments but have seldom been evaluated in relation to chronic health outcomes in 1990–1991 Gulf War veterans. A limited number of Gulf War veteran studies have previously assessed mTBIs in relation to GWI, with varying results. In the Fort Devens cohort, the prevalence of GWI was elevated among veterans who reported a history of three or more mTBIs [52]. TBIs during deployment were infrequently reported in a VA study of 202 Gulf War veterans [53]. There, a history of TBIs overall was associated with symptomatic illness, broadly defined, but not with more narrowly-defined GWI. In addition, two prior studies have reported on effects of mTBI in subsets of the full GWIC

case-control sample evaluated here. The first reported a significant association of mTBIs sustained in theater with GWI [54]. The second identified focal microstructural differences on high order diffusion MRI brain scans among veterans with GWI who reported having mTBIs during deployment, compared to veterans with no mTBI [34].

4.1. Symptom Reporting and GWI Case Definition

Primary GWI case status for the GWIC study was based on the Kansas GWI case criteria [10], with secondary assessment of CMI criteria [8]. Both case definitions were developed in the first decade after the Gulf War and based on the types and pattern of symptoms reported by Gulf War veterans at that time, with Kansas criteria also providing guidelines for excluding veterans with diagnoses that potentially explain their symptoms. By design, GWI cases in our study endorsed significantly more symptoms than controls. However, the frequency of symptoms reported by GWI cases was higher than generally observed in early GWI studies and substantially greater than the symptom burden required to meet Kansas GWI case criteria. This is consistent with other more recent studies of Gulf War veterans that have generally indicated that, over the years, 1990–1991 Gulf War veterans have reported an increasing burden both of chronic symptoms and of diagnosed health conditions [5–7,55,56]. In the current study, this increased symptom burden was also observed in controls, half of whom met CMI criteria.

More than 20 years after the CMI and Kansas GWI case definitions were developed, time and age-associated changes in both the symptom profile and diagnosed conditions affecting Gulf War veterans suggest the likelihood of reduced specificity for both case definitions. This, in turn, would be expected to reduce their utility for accurately characterizing GWI cases for research and other purposes. Increased levels of morbidity observed in this and other studies supports the need for evidence-based revisions to existing GWI case definitions [6,57–59]. A primary consideration is that revised GWI criteria more accurately reflect present day symptoms and diagnosed health conditions associated with service in the 1990–1991 Gulf War in order to optimally distinguish GWI cases from noncases.

4.2. Strengths and Limitations

The GWIC case-control study has several strengths and limitations to consider in assessing research results. Important strengths are the study size and rigorous characterization of veterans for the project, which included a diverse sample of 1990–1991 Gulf War veterans from different regions in the country. To our knowledge, the GWIC multisite project (n = 269) represents the most comprehensive evaluation of clinical, neurological and immune measures of 1990–1991 Gulf War veterans to date and is one of the largest case-control studies conducted in this population. However, the sample included fewer controls than originally targeted for the study, which may prove to be a key limitation in addressing some study questions. In addition, the GWIC study sample was not randomly selected from a defined population, so the extent to which cases and controls are representative of the larger Gulf War veteran population is uncertain.

This initial report from the full GWIC case-control study sample provides an overview of research assessments and general health comparisons between GWI cases and controls. Future papers will report on results of neuroimaging, neurocognitive, immune, and genetic testing from Gulf War veterans evaluated at the three GWIC clinical sites, including the degree to which identified health outcomes differ in GWI subgroups and are associated with exposures during the Gulf War.

5. Conclusions

Limited findings on routine clinical assessment of ill Gulf War veterans underscores the importance of applying multidisciplinary, state of the art research to accelerate progress in addressing GWI.

Improved understanding of brain and immune GWI pathobiology provided by the GWIC and related projects is essential for identifying effective treatments and valid diag-

nostic tests for the complex of serious health problems that continue to affect Gulf War veterans, 30 years after their service in Operation Desert Storm.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/brainsci11091132/s1, Figure S1. GWIC Study Exclusionary Conditions.

Author Contributions: Conceptualization, K.S., N.K. and L.S.; methodology, K.S., N.K., L.S., R.T., M.K., R.K., D.L., P.J. and J.K.C.; formal analysis, L.S., E.Q. and T.H.; investigation, K.S., L.S., N.K., D.L., M.K., R.T., R.K., B.-B.K., A.N.C., J.C., J.A., G.B., V.L., M.A. and K.A.; data curation, E.Q. and T.H.; writing—original draft preparation, L.S. and K.S.; writing—review and editing, N.K., M.K., R.T., D.L., E.Q., T.H., P.J., R.K., B.-B.K., A.N.C., J.C., J.A., G.B., V.L., M.A., K.A. and J.K.C.; funding acquisition K.S., N.K. and L.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Congressionally-Directed Medical Research Program (CDMRP) Gulf War Illness Research Program, U.S. Department of Defense, through CDMRP consortium award W81XWH-13-2-0072 to Kimberly Sullivan at Boston University. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the Department of Defense.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Boards of Boston University (protocol #H-32768, approved 21 May 2014), Miami VA Medical Center (protocol #14/74, approved 5 May 2014), and Baylor College of Medicine (protocol #H-39514, approved 30 September 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Requests for GWIC data and samples can be made through online request from the Boston Biorepository and Integrative Network for Gulf War Illness (BBRAIN) website at http://sites.bu.edu/bbrain/data-request/ (accessed on 25 August 2021).

Acknowledgments: The authors wish to thank all Gulf War veterans who participated in the study. We also thank members of the GWIC Veterans Advisory Board: Denise Nichols, Lynn Santosuosso, Jimmy Arocho, Marylyn Harris, Christine Tron, Timothy Demers, and Frances Perez-Wilhite for their valuable contributions to the project.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. U.S. Department of Defense. *Conduct of the Persian Gulf War—Final Report to Congress*; U.S. Government Printing Office: Washington, DC, USA, 1992.
2. DeFraites, R.F.; Wanat, E.R.I.; Norwood, A.E.; Williams, S.; Cowan, D.; Callahan, T. *Investigation of a Suspected Outbreak of an Unknown Disease among Veterans of Operation Desert Shield/Storm 123d Army Reserve Command, Fort Benjamin Harrison, Indiana, April, 1992*; Water Reed Army Institute of Research: Washington, DC, USA, 1992.
3. Centers for Disease Control and Prevention (CDC). Unexplained illness among Persian Gulf War veterans in an Air National Guard Unit: Preliminary report–August 1990-March 1995. *MMWR Morb. Mortal. Wkly. Rep.* **1995**, *44*, 443–447.
4. Stretch, R.H.; Bliese, P.D.; Marlowe, D.H.; Wright, K.M.; Knudson, K.H.; Hoover, C.H. Physical health symptomatology of Gulf War-era service personnel from the states of Pennsylvania and Hawaii. *Mil. Med.* **1995**, *160*, 131–136. [CrossRef] [PubMed]
5. Sullivan, K.; Krengel, M.; Heboyan, V.; Schildroth, S.; Wilson, C.C.; Iobst, S.; Klimas, N.; Coughlin, S.S. Prevalence and Patterns of Symptoms among Female Veterans of the 1991 Gulf War Era: 25 Years Later. *J. Womens Health* **2020**, *29*, 819–826. [CrossRef] [PubMed]
6. Gifford, E.J.; Vahey, J.; Hauser, E.R.; Sims, K.J.; Efird, J.T.; Dursa, E.K.; Steele, L.; Helmer, D.A.; Provenzale, D. Gulf War illness in the Gulf War Era Cohort and Biorepository: The Kansas and Centers for Disease Control definitions. *Life Sci.* **2021**, *278*, 119454. [CrossRef] [PubMed]
7. Zundel, C.G.; Heeren, T.; Grasso, C.M.; Spiro, I.A.; Proctor, S.P.; Sullivan, K.; Krengel, M. Changes in Health Status in the Ft. Devens Gulf War Veterans Cohort: 1997–2017. *Neurosci. Insights.* **2020**, *15*, 2633105520952675. [CrossRef]
8. Fukuda, K.; Nisenbaum, R.; Stewart, G.; Thompson, W.W.; Robin, L.; Washko, R.M.; Noah, D.L.; Barrett, D.H.; Randall, B.; Herwaldt, B.L.; et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. *JAMA* **1998**, *280*, 981–988. [CrossRef]
9. Kang, H.K.; Mahan, C.M.; Lee, K.Y.; Magee, C.A.; Murphy, F.M. Illnesses among United States veterans of the Gulf War: A population-based survey of 30,000 veterans. *J. Occup. Environ. Med.* **2000**, *42*, 491–501. [CrossRef]
10. Steele, L. 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.* **2000**, *152*, 992–1002. [CrossRef] [PubMed]

11. Research Advisory Committee on Gulf War Veterans' Illnesses. *Gulf War Illness and the Health of Gulf War Veterans: Scientific Findings and Recommendations*; U.S. Government Printing Office: Washington, DC, USA, 2008; 2008-560-653.
12. Haley, R.W.; Kurt, T.L. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study. *JAMA* **1997**, *277*, 231–237. [CrossRef]
13. Nisenbaum, R.; Barrett, D.H.; Reyes, M.; Reeves, W.C. Deployment stressors and a chronic multisymptom illness among Gulf War veterans. *J. Nerv. Ment. Dis.* **2000**, *188*, 259–266. [CrossRef]
14. Cherry, N.; Creed, F.; Silman, A.; Dunn, G.; Baxter, D.; Smedley, J.; Taylor, S.; Macfarlane, G.J. Health and exposures of United Kingdom Gulf war veterans. Part II: The relation of health to exposure. *Occup. Environ. Med.* **2001**, *58*, 299–306. [CrossRef] [PubMed]
15. Steele, L.; Sastre, A.; Gerkovich, M.M.; Cook, M.R. Complex factors in the etiology of Gulf War illness: Wartime exposures and risk factors in veteran subgroups. *Environ. Health Perspect.* **2012**, *120*, 112–118. [CrossRef] [PubMed]
16. White, R.F.; Steele, L.; O'Callaghan, J.P.; Sullivan, K.; Binns, J.H.; Golomb, B.A.; Bloom, F.E.; Bunker, J.A.; Crawford, F.; Graves, J.C.; et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: Effects of toxicant exposures during deployment. *Cortex* **2016**, *74*, 449–475. [CrossRef] [PubMed]
17. Krengel, M.; Sullivan, K.; Heboyan, V.; Zundel, C.G.; Wilson, C.C.; Klimas, N.; Coughlin, S.S. Neurotoxicant exposures and rates of Chronic Multisymptom Illness and Kansas Gulf War illness criteria in Gulf War deployed women veterans. *Life Sci.* **2021**, *280*, 119623. [CrossRef]
18. Zhang, Q.; Zhou, X.D.; Denny, T.; Ottenweller, J.E.; Lange, G.; LaManca, J.J.; Lavietes, M.H.; Pollet, C.; Gause, W.C.; Natelson, B.H. Changes in immune parameters seen in Gulf War veterans but not in civilians with chronic fatigue syndrome. *Clin. Diagn. Lab. Immunol.* **1999**, *6*, 6–13. [CrossRef]
19. Vojdani, A.; Thrasher, J.D. Cellular and humoral immune abnormalities in Gulf War veterans. *Environ. Health Perspect.* **2004**, *112*, 840–846. [CrossRef]
20. Heaton, K.J.; Palumbo, C.L.; Proctor, S.P.; Killiany, R.J.; Yurgelun-Todd, D.A.; White, R.F. Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *Neurotoxicology* **2007**, *28*, 761–769. [CrossRef]
21. Whistler, T.; Fletcher, M.A.; Lonergan, W.; Zeng, X.R.; Lin, J.M.; Laperriere, A.; Vernon, S.D.; Klimas, N.G. Impaired immune function in Gulf War Illness. *BMC Med. Genom.* **2009**, *2*, 12. [CrossRef]
22. Chao, L.L.; Abadjian, L.; Hlavin, J.; Meyerhoff, D.J.; Weiner, M.W. Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: A study at 4T. *Neurotoxicology* **2011**, *32*, 814–822. [CrossRef]
23. Janulewicz, P.A.; Krengel, M.H.; Maule, A.; White, R.F.; Cirillo, J.; Sisson, E.; Heeren, T.; Sullivan, K. Neuropsychological characteristics of Gulf War illness: A meta-analysis. *PLoS ONE* **2017**, *12*, e0177121. [CrossRef]
24. Sullivan, K.; Krengel, M.; Bradford, W.; Stone, C.; Thompson, T.A.; Heeren, T.; White, R.F. Neuropsychological functioning in military pesticide applicators from the Gulf War: Effects on information processing speed, attention and visual memory. *Neurotoxicol. Teratol.* **2018**, *65*, 1–13. [CrossRef] [PubMed]
25. Zhang, Y.; Avery, T.; Vakhtin, A.A.; Mathersul, D.; Tranvinh, E.; Wintermark, M.; Massaband, P.; Ashford, J.W.; Bayley, P.J.; Furst, A.J. Brainstem atrophy in Gulf War Illness. *Neurotoxicology* **2020**, *78*, 71–79. [CrossRef]
26. Abou-Donia, M.B.; Wilmarth, K.R.; Jensen, K.F.; Oehme, F.W.; Kurt, T.L. Neurotoxicity resulting from coexposure to pyridostigmine bromide, DEET, and permethrin: Implications of Gulf War chemical exposures. *J. Toxicol. Environ. Health* **1996**, *48*, 35–56. [CrossRef] [PubMed]
27. Zakirova, Z.; Tweed, M.; Crynen, G.; Reed, J.; Abdullah, L.; Nissanka, N.; Mullan, M.; Mullan, M.J.; Mathura, V.; Crawford, F.; et al. Gulf War agent exposure causes impairment of long-term memory formation and neuropathological changes in a mouse model of Gulf War Illness. *PLoS ONE* **2015**, *10*, e0119579. [CrossRef] [PubMed]
28. Hattiangady, B.; Mishra, V.; Kodali, M.; Shuai, B.; Rao, X.; Shetty, A.K. Object location and object recognition memory impairments, motivation deficits and depression in a model of Gulf War illness. *Front. Behav. Neurosci.* **2014**, *8*, 78. [CrossRef] [PubMed]
29. Lacagnina, M.J.; Li, J.; Lorca, S.; Rice, K.C.; Sullivan, K.; O'Callaghan, J.P.; Grace, P.M. A role for neuroimmune signaling in a rat model of Gulf War Illness-related pain. *Brain Behav. Immun.* **2021**, *91*, 418–428. [CrossRef] [PubMed]
30. Michalovicz, L.T.; Kelly, K.A.; Sullivan, K.; O'Callaghan, J.P. Acetylcholinesterase inhibitor exposures as an initiating factor in the development of Gulf War Illness, a chronic neuroimmune disorder in deployed veterans. *Neuropharmacology* **2020**, *171*, 108073. [CrossRef] [PubMed]
31. Weathers, F.W.; Bovin, M.J.; Lee, D.J.; Sloan, D.M.; Schnurr, P.P.; Kaloupek, D.G.; Keane, T.M.; Marx, B.P. The Clinician-Administered PTSD Scale for DSM-5 (CAPS-5): Development and initial psychometric evaluation in military veterans. *Psychol. Assess.* **2018**, *30*, 383–395. [CrossRef] [PubMed]
32. First, M.B.; Williams, J.B.W.; Karg, R.S.; Spitzer, R.L. *Structured Clinical Interview for DSM-5*; American Psychiatric Association: Arlington, VA, USA, 2015.
33. Guan, Y.; Cheng, C.H.; Chen, W.; Zhang, Y.; Koo, S.; Krengel, M.; Janulewicz, P.; Toomey, R.; Yang, E.; Bhadelia, R.; et al. Neuroimaging Markers for Studying Gulf-War Illness: Single-Subject Level Analytical Method Based on Machine Learning. *Brain Sci.* **2020**, *10*, 884. [CrossRef]

34. Cheng, C.H.; Koo, B.B.; Calderazzo, S.; Quinn, E.; Aenlle, K.; Steele, L.; Klimas, N.; Krengel, M.; Janulewicz, P.; Toomey, R.; et al. Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. *Brain Behav. Immun.* **2020**, *89*, 281–290. [CrossRef]
35. Kazis, L.E. The Veterans SF-36® Health Status Questionnaire: Development and Application in the Veterans Health Administration. *Medical. Outcomes Trust Monit.* **2000**, *5*, 1–14.
36. Melzack, R. The McGill Pain Questionnaire: Major properties and scoring methods. *Pain* **1975**, *1*, 277–299. [CrossRef]
37. Smets, E.M.; Garssen, B.; Bonke, B.; De Haes, J.C. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *J. Psychosom. Res.* **1995**, *39*, 315–325. [CrossRef]
38. Buysse, D.J.; Reynolds, C.F., 3rd; Monk, T.H.; Berman, S.R.; Kupfer, D.J. The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Res.* **1989**, *28*, 193–213. [CrossRef]
39. Proctor, S.P. *Development of a Structured Neurotoxicant Assessment Checklist (SNAC) for Clinical Use in Veteran Populations*; Department of Veterans Affairs, VA Boston Healthcare System: Boston, MA, USA. Available online: https://www.va.gov/RAC-GWVI/meetings/sep2014/Presentation7KimberlySullivanNancyKlimas.pdf (accessed on 25 August 2021).
40. Robbins, C.A.; Daneshevar, D.H.; Picano, J.D.; Gevett, B.E.; Baugh, C.M.; Riley, D.O.; Nowinski, C.J.; McKee, A.C.; Cantu, R.C.; Stern, R.A. Self-reported concussion history: Impact of providing a definition of concussion. *Open Access J. Sports Med.* **2014**, *7*, 99–103. [CrossRef] [PubMed]
41. Satterthwaite, F.W. An approximate distribution of estimates of variance components. *Biom. Bull.* **1946**, *2*, 110–114. [CrossRef]
42. Shuster, J.J. Student t-tests for potentially abnormal data. *Stat. Med.* **2009**, *28*, 2170–2184. [CrossRef] [PubMed]
43. *SAS Software (v.9.4)*; SAS Institute Inc.: Cary, NC, USA, 2020.
44. Wolfe, F.; Smythe, H.A.; Yunus, M.B.; Bennett, R.M.; Bombardier, C.; Goldenberg, D.L.; Tugwell, P.; Campbell, S.M.; Abeles, M.; Clark, P.; et al. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum.* **1990**, *33*, 160–172. [CrossRef] [PubMed]
45. Marmot, M.G.; Smith, G.D.; Stansfeld, S.; Patel, C.; North, F.; Head, J.; White, I.; Brunner, E.; Feeney, A. Health inequalities among British civil servants: The Whitehall II study. *Lancet* **1991**, *337*, 1387–1393. [CrossRef]
46. Eisen, S.A.; Kang, H.K.; Murphy, F.M.; Blanchard, M.S.; Reda, D.J.; Henderson, W.G.; Toomey, R.; Jackson, L.W.; Alpern, R.; Parks, B.J.; et al. Gulf War Study Participating Investigators. Gulf War veterans' health: Medical evaluation of a U.S. cohort. *Ann. Intern. Med.* **2005**, *142*, 881–890. [CrossRef] [PubMed]
47. Gean, E.G.; Ayers, C.K.; Winchell, K.A.; Freeman, M.; Press, A.M.; Paynter, R.; Kansagara, D.; Nugent, S.M. Biological measures and diagnostic tools for Gulf War Illness—A systematic review. *Life Sci.* **2021**, *275*, 119360. [CrossRef]
48. Richardson, R.D.; Engel, C.C., Jr.; McFall, M.; McKnight, K.; Boehnlein, J.K.; Hunt, S.C. Clinician attributions for symptoms and treatment of Gulf War-related health concerns. *Arch. Intern. Med.* **2001**, *161*, 1289–1294. [CrossRef] [PubMed]
49. Anastasides, N.; Chiusano, C.; Gonzalez, C.; Graff, F.; Litke, D.R.; McDonald, E.; Presnall-Shvorin, J.; Sullivan, N.; Quigley, K.S.; Pigeon, W.R.; et al. Helpful ways providers can communicate about persistent medically unexplained physical symptoms. *BMC Fam. Pract.* **2019**, *20*, 13. [CrossRef] [PubMed]
50. Kaimal, G.; Dieterich-Hartwell, R. Grappling with Gulf War Illness: Perspectives of Gulf War Providers. *Int. J. Environ. Res. Public Health* **2020**, *17*, 8574. [CrossRef]
51. Sullivan, K.; Krengel, M.; Proctor, S.P.; Devine, S.; Heeren, T.; White, R.F. Cognitive functioning in treatment-seeking Gulf War veterans: Pyridostigmine bromide use and PTSD. *J. Psychopathol. Behav. Assess.* **2003**, *25*, 95–103. [CrossRef]
52. Yee, M.K.; Janulewicz, P.A.; Seichepine, D.R.; Sullivan, K.A.; Proctor, S.P.; Krengel, M.H. 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 Sci.* **2017**, *7*, 79. [CrossRef] [PubMed]
53. Chao, L.L. The Relationship between Traumatic Brain Injury and Rates of Chronic Symptomatic Illness in 202 Gulf War Veterans. *Mil. Med.* **2018**, *183*, e571–e579. [CrossRef] [PubMed]
54. Janulewicz, P.; Krengel, M.; Quinn, E.; Heeren, T.; Toomey, R.; Killiany, R.; Zundel, C.; Ajama, J.; O'Callaghan, J.; Steele, L.; et al. The Multiple Hit Hypothesis for Gulf War Illness: Self-Reported Chemical/Biological Weapons Exposure and Mild Traumatic Brain Injury. *Brain Sci.* **2018**, *8*, 198. [CrossRef]
55. Porter, B.; Long, K.; Rull, R.P.; Dursa, E.K.; Millennium Cohort Study Team. Prevalence of Chronic Multisymptom Illness/Gulf War Illness over Time among Millennium Cohort Participants, 2001 to 2016. *J. Occup. Environ. Med.* **2020**, *62*, 4–10. [CrossRef] [PubMed]
56. Fappiano, C.M.; Baraniuk, J.N. Gulf War Illness Symptom Severity and Onset: A Cross-Sectional Survey. *Mil. Med.* **2020**, *185*, e1120–e1127. [CrossRef]
57. National Academy of Sciences, Institute of Medicine. *Chronic Multisymptom Illness in Gulf War Veterans: Case Definitions Reexamined*; National Academies Press: Washington, DC, USA, 2014.
58. Research Advisory Committee on Gulf War Veterans' Illnesses. *Gulf War Illness and the Health of Gulf War Veterans: Research Update and Recommendations, 2009–2013*; U.S. Government Printing Office: Washington, DC, USA, 2014.
59. U.S. Department of Veterans Affairs Office of Research & Development. Gulf War Research Strategic Plan 2013–2017. Available online: https://www.research.va.gov/pubs/docs/gwresearch-strategicplan.pdf (accessed on 25 August 2021).

Life Sciences xxx (xxxx) 119903

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Boston biorepository, recruitment and integrative network (BBRAIN): A resource for the Gulf War Illness scientific community

D. Keating [a], C.G. Zundel [b], M. Abreu [c, d], M. Krengel [e], K. Aenlle [c, d], D. Nichols [a, 1], R. Toomey [f], L.L. Chao [g], J. Golier [h, i], L. Abdullah [j, k, l], E. Quinn [m], T. Heeren [m], J.R. Groh [b], B.B. Koo [n], R. Killiany [a, n], M.L. Loggia [o], J. Younger [p], J. Baraniuk [q], P. Janulewicz [a], J. Ajama [a], M. Quay [a], P.W. Baas [r], L. Qiang [r], L. Conboy [s], E. Kokkotou [t], J.P. O'Callaghan [u], L. Steele [u], N. Klimas [c, d], K. Sullivan [a, *]

[a] Boston University School of Public Health, Department of Environmental Health, 715 Albany St. T4W, Boston, MA 02118, USA
[b] Boston University School of Medicine, Behavioral Neuroscience Program, 72 East Concord St., Boston, MA 02118, USA
[c] Dr. Kiran C. Patel College of Osteopathic Medicine, Institute for Neuroimmune Medicine, Nova Southeastern University, Fort Lauderdale, FL 33314, USA
[d] Geriatric Research Education and Clinical Center, Miami VA Medical Center, Miami, FL 33125, USA
[e] Boston University School of Medicine, Department of Neurology, 72 East Concord St., Boston, MA 02118, USA
[f] Department of Psychological and Brain Sciences, College of Arts and Sciences, Boston University, 900 Commonwealth Ave., Boston, MA, USA
[g] San Francisco Veterans Affairs Health Care System, University of California, San Francisco, CA 94143, USA
[h] James J. Peters VA Medical Center, OOMH-526, 130 West Kingsbridge Road, Bronx, NY 10468, USA
[i] Psychiatry Department, Icahn School of Medicine at Mount Sinai, 1428 Madison Ave, New York, NY 10029, USA
[j] Roskamp Institute, 2040 Whitfield Ave, Sarasota, FL 34243, USA
[k] Open University, Milton Keynes, United Kingdom
[l] James A. Haley Veterans' Hospital, Tampa, FL, USA
[m] Boston University School of Public Health, Department of Biostatistics, 715 Albany St., Boston, MA 02118, USA
[n] Boston University School of Medicine, Department of Anatomy and Neurobiology, 72 East Concord St., Boston, MA 02118, USA
[o] Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA
[p] Neuroinflammation, Pain & Fatigue Lab, University of Alabama at Birmingham, Birmingham, AL, USA
[q] Department of Medicine, Georgetown University, Washington, DC, USA
[r] Drexel University College of Medicine, Department of Neurobiology and Anatomy, 2900 Queen Lane, Philadelphia, PA 19129, USA
[s] Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115, USA
[t] Health Effects Laboratory Division, Centers for Disease Control and Prevention National Institute for Occupational Safety and Health, Morgantown, WV, USA
[u] Baylor College of Medicine Neuropsychiatry Division, Department of Psychiatry and Behavioral Sciences, Houston, TX 77030, USA

ARTICLE INFO

Keywords:
BBRAIN
GWI
GWIC
Gulf war
Gulf war illness

ABSTRACT

Aims: Gulf War Illness (GWI), a chronic debilitating disorder characterized by fatigue, joint pain, cognitive, gastrointestinal, respiratory, and skin problems, is currently diagnosed by self-reported symptoms. The Boston Biorepository, Recruitment, and Integrative Network (BBRAIN) is the collaborative effort of expert Gulf War Illness (GWI) researchers who are creating objective diagnostic and pathobiological markers and recommend common data elements for GWI research.

Main methods: BBRAIN is recruiting 300 GWI cases and 200 GW veteran controls for the prospective study. Key data and biological samples from prior GWI studies are being merged and combined into retrospective datasets.

Abbreviations: BBRAIN, Boston Biorepository, Recruitment and Integrative Network for Gulf War Illness; GW, Gulf War; GWI, Gulf War Illness; GWIC, Gulf War Illness Consortium; VA, Veterans Affairs

* Corresponding author at: Boston University School of Public Health, 715 Albany Street, T4W, Boston, MA 02118, USA.
E-mail addresses: dmk13@bu.edu (D. Keating), cgzundel@bu.edu (C.G. Zundel), mhk@bu.edu (M. Krengel), toomey@bu.edu (R. Toomey), linda.chao@ucsf.edu (L.L. Chao), julia.golier@va.gov (J. Golier), labdullah@roskampinstitute.org (L. Abdullah), eq@bu.edu (E. Quinn), tch@bu.edu (T. Heeren), jengroh@bu.edu (J.R. Groh), bbkoo@bu.edu (B.B. Koo), killiany@bu.edu (R. Killiany), marco.loggia@mgh.harvard.edu (M.L. Loggia), younger@uab.edu (J. Younger), baraniuj@georgetown.edu (J. Baraniuk), paj@bu.edu (P. Janulewicz), quaym@bu.edu (M. Quay), pwb22@drexel.edu (P.W. Baas), lq24@drexel.edu (L. Qiang), lisa_conboy@hms.haravard.edu (L. Conboy), ekokkoto@bidmc.harvard.edu (E. Kokkotou), jdo5@cdc.gov (J.P. O'Callaghan), Lea.Steele@bcm.edu (L. Steele), tty@bu.edu (K. Sullivan).
[1] US Air Force retired.

https://doi.org/10.1016/j.lfs.2021.119903
Received 28 February 2021; Received in revised form 31 July 2021; Accepted 17 August 2021
0024-3205/

They will be made available for data mining by the BBRAIN network and the GWI research community. Prospective questionnaire data include general health and chronic symptoms, demographics, measures of pain, fatigue, medical conditions, deployment and exposure histories. Available repository biospecimens include blood, plasma, serum, saliva, stool, urine, human induced pluripotent stem cells and cerebrospinal fluid.
Key findings: To date, multiple datasets have been merged and combined from 15 participating study sites. These data and samples have been collated and an online request form for repository requests as well as recommended common data elements have been created. Data and biospecimen sample requests are reviewed by the BBRAIN steering committee members for approval as they are received.
Significance: The BBRAIN repository network serves as a much needed resource for GWI researchers to utilize for identification and validation of objective diagnostic and pathobiological markers of the illness.

1. Introduction

Gulf War Illness (GWI) is a debilitating, chronic, multi-symptom disorder affecting nearly one-third of veterans in the 1991 Gulf War (GW) [1,2]. The illness is characterized by debilitating fatigue, chronic pain, cognitive dysfunction, headaches, respiratory problems, and gastrointestinal disturbances [3–5]. Veterans suffering from GWI can experience significant impairment in their daily activities and quality of life. Despite promising recent research in correlating biomarkers to GWI symptoms, GWI primarily remains diagnosed by self-report. The study of potential diagnostic biomarkers to date has not been supported by larger sample sizes and has not been validated in other cohorts [6–11]. Basing diagnosis on self-reported symptoms makes treatment development and access to care for GW veterans persistently difficult. There is a critical need for an objective diagnostic test for GWI to alleviate GW veterans' difficulties with obtaining service-related benefits and validation of their symptoms and for use as primary outcome measures for treatment trials.

"A biorepository is an entity that receives, processes, stores, and/or disseminates biospecimens, their derivatives, and relevant data, as needed [12]. It encompasses the physical location and the full range of activities associated with its operation [12]." A recent review by Garcia et al., showed that rare disease biobanks have the ability to identify and validate genetic and omics biomarkers as well as inform treatment development strategies for these rare disorders [13]. However, it was also noted that many of these repositories lacked the corresponding clinical outcomes data needed to make the biomarker samples most useful for correlation with the disease symptoms.

Therefore, the need is clear for a biorepository network of freely sharing biospecimens with corresponding comprehensive clinical outcomes data in the field of GWI research. There is also a need for retrospective data mining from prior studies that are hard to replicate (i.e., cerebrospinal fluid, PET, and MRI brain imaging outcomes). These clinical outcomes that are common (common data elements) among the different prior studies provide power to document differences that might not emerge in the smaller sample cohorts. These common data elements are also needed to ensure comparability of study results, particularly for treatment trial efficacy testing [14,15].

The Boston Biorepository, Recruitment and Integrative Network (BBRAIN) for GWI was designed to serve as a resource for the GWI research community to hasten biomarker discovery and validate prior results in a well-characterized cohort of GW veterans. The BBRAIN study was built upon and incorporates the already existing Boston GWI consortium [16]. The GWI consortium brought together leading experts from different fields into the GWI research community. Since its conception, the GWI consortium has established an extensive multi-site data set with cognitive measures, brain imaging, health symptom data, and biorepository blood and saliva specimens for several hundred GW veterans. GWIC has greatly expanded the field's ability to explore and identify specific 'objective' biomarkers and 'personalized' treatment strategies for veterans with GWI by utilizing a small biorepository shared with the GWI research community, resulting in 20 additional federally funded studies. This lead to 34 biomarker publications of lipidomic, proteomic, epigenetic, genetic susceptibility, mitochondrial, CNS autoantibodys, and tau markers in clinical and preclinical translational studies [7,11,17–43]. It also resulted in funding to establish two additional consortia, including BBRAIN and the Gulf War Illness Clinical Trials Consortium [14]. Since its inception in 2018, BBRAIN has built upon this existing infrastructure at Boston University and 14 other participating sample and data resource sites to establish a much-needed resource for the GWI research community that is available for data and sample sharing.

2. Methods: BBRAIN structure

2.1. Leadership

The lead site of BBRAIN is at Boston University School of Public Health and makes up the network coordinating center. The network coordinating center staff members have diverse expertise in neuropsychology, brain imaging, exposure assessment, data management, statistical programming and study operations. These skills are integral for maintaining a multi-site biorepository and promoting collaboration within the GWI research field.

2.2. Participating sites

The BBRAIN collaboration brings together leading investigators from 15 institutions to support participant recruitment, administrative activities, data management and biostatistics, and biorepository and biomarker evaluation. The BBRAIN collaboration is composed of the network coordinating center, steering committee, retrospective resource sites and four prospective resource sites (Fig. 1). The four resource sites where prospective subject recruitment is taking place include Boston University School of Public Health, Miami VA Medical Center and Nova Southeastern University, Bronx VA Medical Center and the San Francisco VA Medical Center.

2.2.1. Steering committee

Oversight of the BBRAIN is coordinated by a Steering Committee made up of the BBRAIN PI, the resource site PIs, the network coordinating directors, and the consumer advocate. The Steering Committee monitors research site performance and determines individual study performance. The Steering Committee is also responsible for establishing standard operating procedures, utilizing ISBER Best Practices for Biorepositories, and utilizing BUSPH criteria templates for Data Use Agreements across sites and institutions sharing samples and data [12]. Researchers interested in obtaining BBRAIN samples can apply to the Steering Committee. The group will decide on the appropriateness (i.e., for GWI research and not redundant with ongoing research) and priority of sharing samples on a case-by-case basis.

2.2.2. Network coordinating center

The network coordinating center for the biorepository is responsible for overseeing IRB protocol and regulatory submissions and approvals, establishing standard protocols across all sites, and conducting data management and monitoring while ensuring study participant confidentiality. The center is led by the BBRAIN study PI at BUSPH and sup-

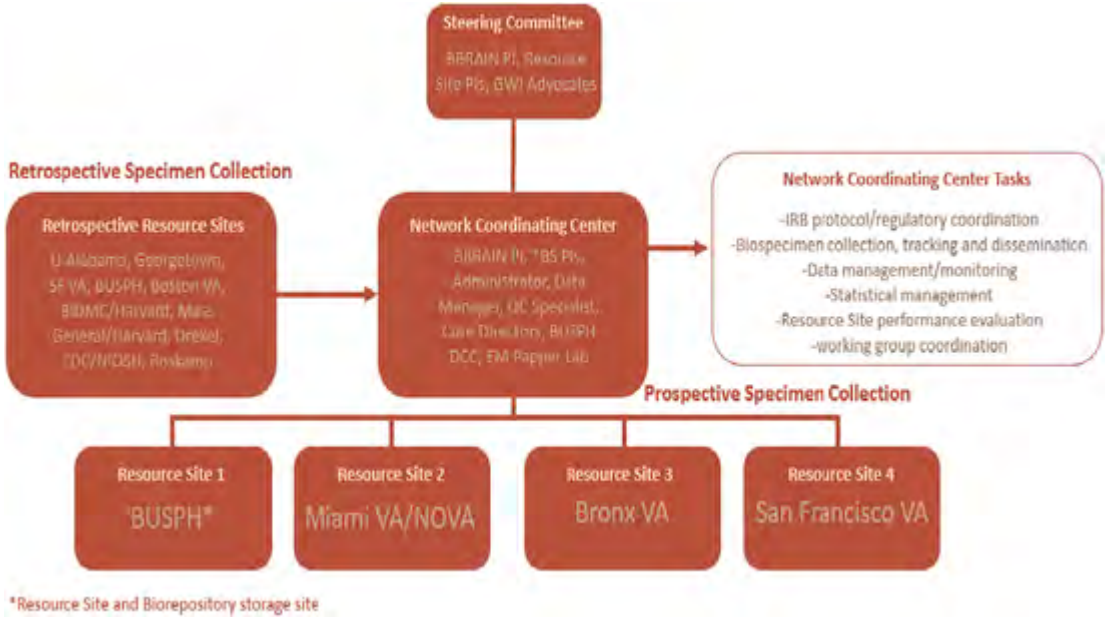


Fig. 1. BBRAIN organizational structure.

ported by faculty and administrative staff at BUSPH. The network coordinating center provides support and coordination for prospective data collection of demographic surveys, cognitive test data, serum, plasma, saliva, stool, and urine samples from 500 GW veteran study participants. Additionally, the network coordinating center serves as a gatekeeper for requests for repository site sharing and coordinates approvals with the steering committee members in consultation with the biorepository contributors. A virtual biorepository is established using laboratory software LDMS (Frontier and/or FreezerPro for resource sites). Resource sites send newly obtained biospecimens and data to laboratory storage facilities at Nova Southeastern University and Boston University Medical Campus (BUMC) as the prospective repository is being created.

2.2.2.1. Subject Confidentiality. As in all human subject research, protecting subject confidentiality is imperative. For retrospective data, all samples and data are de-identified. For prospective data collection, participants' contact information is kept in a study-specific electronic capture web-based platform on a secure server, including multiple password protection layers that are only accessible by approved study team members. All source documents are kept in a locked cabinet, and all data is behind password-protected and encrypted devices. Samples that are shipped are labeled with a unique identifier code for tracking purposes within the biorepository. Participants are made aware of the confidentiality measure taken at the time of the phone screener and again during the consent process and are consented to share their coded study samples and data for the repository and other future GWI related studies. All data and samples that are shared from the repository are coded with no individual identifiers.

2.2.3. Retrospective resource sites

One of the BBRAIN's primary objectives is to establish a retrospective biorepository network by data mining from existing BBRAIN collaborators' stored specimens, cognitive data, and brain imaging data from study participants who have consented to share these data and samples for future studies in a de-identified manner. Retrospective resource sites have already provided some stored specimens from prior studies with GW veterans cataloged and made available for within Network and outside of Network investigators. Common data element datasets from these prior studies are also being created to improve power for new analyses. Currently, available samples include blood serum (n = 300), plasma (n = 1100), peripheral blood mononuclear cells (PBMCs n = 600), DNA (n = 600), human-induced pluripotent stem cells (n = 9), cerebrospinal fluid (n = 150), cognitive data (n = 400), brain imaging data (see Fig. 3 range n = 50-280) and corresponding demographic/survey data from retrospective resource sites, including University of Alabama at Birmingham, San Francisco VA, Harvard / Beth Israel Deaconess Medical Center (BIDMC), Georgetown University, Boston University and Drexel University (Fig. 2) [38]. Brain imaging data includes MRI volumetric and diffusion tensor imaging, MR spectroscopy, functional MRI and positron emission tomography (PET) imaging with peripheral benzodiazepine receptor [^{11}C]-PBR28 and fluorodeoxyglucose 18F-FDG tracers [7,10,11,44,45]. Although some of these samples have been stored and processed differently, these details will be made available to the requesting research investigators to meet their study needs during a study consultation. In addition, preclinical animal retrospective data and tissue samples are also available for sharing upon request from the CDC/NIOSH and Roskamp Institute GWI animal models. As previously mentioned, data collection for the biorepository is coordinated and quality checked by the network coordinating center.

2.2.4. Prospective resource sites

Subject recruitment for the prospective study is conducted at four prospective sites comprising of Boston University School of Public

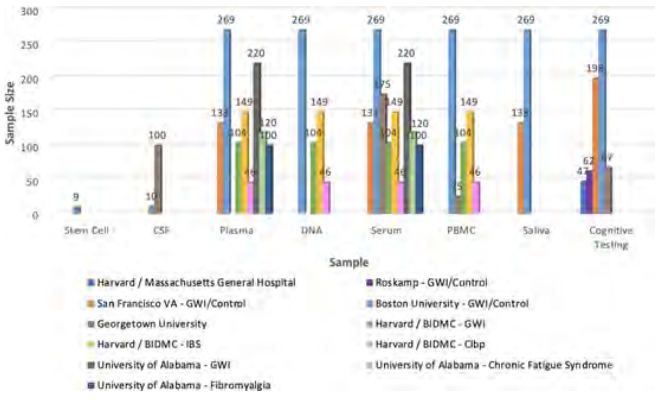


Fig. 2. Retrospective BBRAIN Samples and Data Repository.

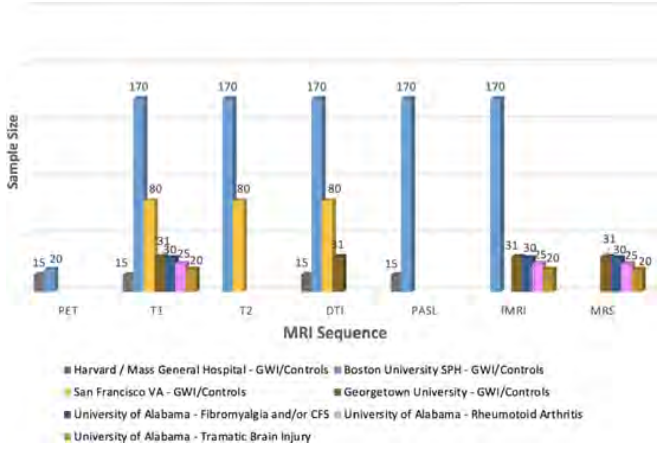


Fig. 3. BBRAIN MRI and PET Imaging Repository.

Health (BUSPH), Miami VA Medical Center / Nova Southeastern University (NSU), Bronx VA Medical Center and San Francisco VA Medical Center (Fig. 1). These sites were chosen due to their access to established GW veteran cohorts and important prior research contributions. Data from the prospective resource sites is being added to the BBRAIN biorepository as subject recruitment accrues.

3. Prospective study methods

3.1. Participants

This case-control study is recruiting 500 GW veterans encompassing 300 GWI cases and 200 GW veteran controls. GWI cases are determined by the Kansas GWI criteria and the four recruitment sites are oversampling women veterans from their prior cohorts [4]. Although not exclusion criteria, smoking history, medication use, and other demographic and health outcomes are being collected and available to requesting investigators.

3.2. Inclusion/exclusion criteria

Study eligibility includes deployment to the Persian Gulf in the 1990-1991 Gulf War without any medical exclusions required for participation. To meet case criteria, the individual needs to endorse symptoms in three of six health symptom domains: pain, fatigue, neurological/cognitive/mood, skin, gastrointestinal, and respiratory [4]. If an individual does not meet the Kansas criteria and has no exclusionary conditions, they are categorized as a control. The criteria for prospective study participants is determined by using the Kansas GWI case definition [4]. Veterans are excluded from being considered GWI cases or controls for the Kansas criteria if they report being diagnosed by a physician with medical or psychiatric conditions that would otherwise account for their symptoms or interfere with their ability to report their symptoms. The Kansas exclusion criteria encompass conditions such as diabetes, heart disease other than hypertension, stroke, lupus, multiple sclerosis, cancer, liver disease, chronic infection, or serious brain injury. Veterans are also excluded if they report being diagnosed with schizophrenia or bipolar disorder or if they have been hospitalized in the past 5 years for alcohol/drug dependence, depression, or post-traumatic stress disorder (PTSD). Potential participants are screened by telephone to determine whether they meet inclusionary or exclusionary criteria for study participation [4]. Additionally, during the phone screen eligible participants are categorized as a case or control based on the Kansas GWI case criteria [4]. Although Kansas criteria are primarily used for comparing study outcomes, the CDC chronic multi-symptom illness case criteria are also obtained for all study participants [5]. These criteria include symptoms in two out of three symptom domains including fatigue, mood-cognition and pain [5].

3.3. Methods

The study protocol for the prospective resource site clinical case-control study consists of five components:

1) Neuropsychological testing: Measures from a previously validated assessment of cognitive function and common data elements in GW veterans are included to assess cognitive outcomes [15,35,46–48]. The neuropsychological test battery assesses the functional domains of attention and executive abilities, psychomotor function, visuospatial skills, memory, general intellectual abilities and mood. The battery includes tests shown to have high specificity and sensitivity for detecting changes in neuropsychological functions between veterans with and without GWI and which were

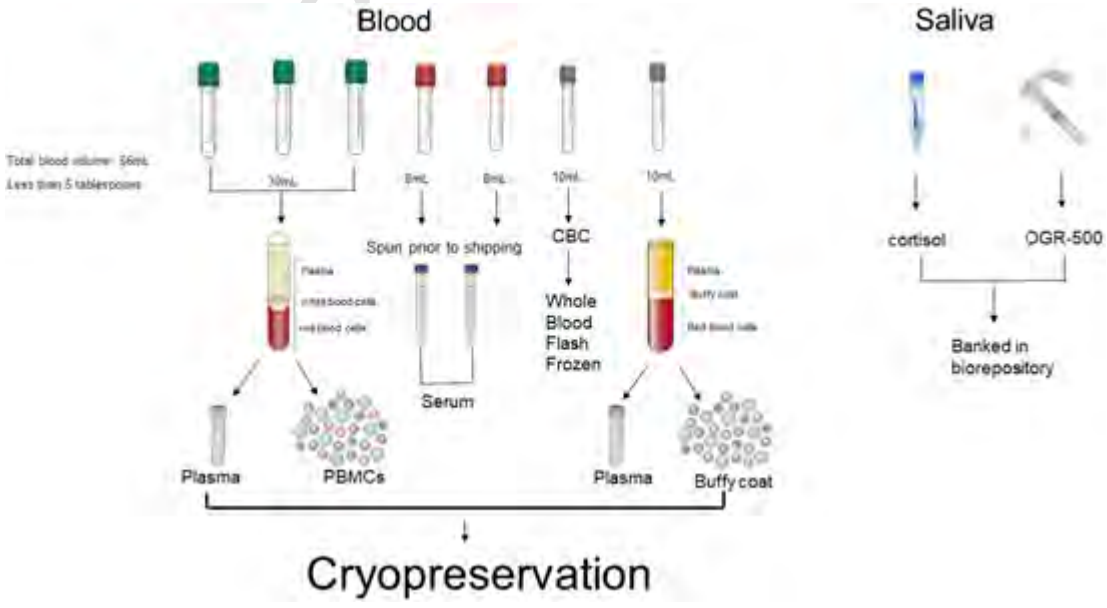


Fig. 4. Blood and saliva aliquot scheme.

recommended to be used across studies as common data elements [35,47].

2) Surveys: The set of surveys administered were all included in the GWI common data elements and collect data on health symptoms, neurotoxicant exposures, mood and quality of life. Clinical assessments include the Pittsburgh Sleep Quality Index (PSQI), Visual Analog Scale (VAS) for pain, Kansas Gulf War and Health Questionnaire, Multidimensional Fatigue Inventory (MFI-20) questionnaire, MOS Short Form 36-veteran version (SF-36V), and Profile of Mood States (POMS), as well as the medical conditions checklist. Additional surveys that were also included in the exposure assessment common data elements include the Kansas Gulf War Experiences and Exposures Questionnaire and the Structured Neurotoxicant Assessment Checklist (SNAC) [49–54].
3) Blood draw: Approximately 79 mL of blood are drawn from the participant for local lab clinical testing, immune biomarker lab testing, and biorepository storage. Fasting blood samples are collected by venipuncture by a trained phlebotomist in the morning. A small amount of blood is analyzed at the local labs for complete blood count, lipid panels, thyroid stimulating hormone, antinuclear antibodies, and rheumatoid factor. The remaining blood samples for each participant are shipped to Nova Southeastern University for sample processing and storage in the biorepository. Blood samples are analyzed to measure plasma cytokine levels, complete blood count, and RNA extraction is performed from PBMCs collected. PBMCs isolated from sodium heparin and EDTA tubes are stored in liquid nitrogen for cryopreservation. RNA isolated from PBMCs is aliquoted and stored at -80 °C. All data from these analyses will be made available as part of the biorepository. Whole blood and blood derivatives such as serum, heparin and EDTA plasma are prepared at various volume aliquots and frozen at -80 °C for storage.
4) Saliva sample: Approximately 6 mL of saliva are collected throughout the study visit. These samples are collected at four different time points: after the participant consent form is signed, after the blood draw and physical exam, after completion of surveys, and at the end of the visit. Three of the collections are performed when the participant is in a fasting state. These samples are used to measure salivary cortisol levels and are stored in a -80 °C freezer. An OGR-600 tube for saliva sample collection is also performed upon fasting for DNA analysis and stored at room temperature before shipping to EM Papper Lab at NOVA Southeastern University. All saliva samples are batch shipped to Nova Southeastern University for planned assessment, cortisol analyses and DNA extraction. The remaining saliva samples are also aliquoted down and frozen for biorepository sample requests. Cortisol and DNA will be made available in the biorepository.

3.3.1. Sample processing methods

Blood samples are shipped on the day of collection at room temperature overnight to the EM Papper laboratory at Nova Southeastern University. As previously mentioned, saliva samples are batch shipped on dry ice, from storage at each prospective site's local storage location. Samples received by the biorepository laboratory at Nova Southeastern University are processed within 2 h of delivery. This includes an immediate quality check of the condition of sample tubes, the outer and inner packaging, and the associated chain of custody that accompanies the sample. Acceptable samples are then accessioned by the biorepository team and entered into the Laboratory Data Management System. This allows for the tracking of barcoded aliquots from each phase of the process, including storage conditions and requirements, and will allow expeditious processing of sample requests. Primary tubes are processed to isolate the blood derivatives: plasma, serum, PBMCs, buffy coat, and red blood cell pellets. Aliquots will be created per the aliquot scheme (see Fig. 4) and tracked for their temperature and location. Each of these blood derivative type are stored at its optimal temperature per standard protocol for the EM Papper laboratory at Nova Southeastern University. Specific blood processing methods are listed below.

3.3.1.1. Separation of plasma from the cellular fraction. Whole blood samples are processed as described below to obtain a buffy coat fraction and plasma for cryopreservation. In the area designated for processing blood, the whole blood (collected in tubes containing an anticoagulant such as ethylene-diamineteraaceticacid-EDTA or Heparin) is fractionated by centrifuging at 2000 x g for 10 min at room temperature. This separates the blood into three visible layers (Fig. 4). The upper layer, the plasma layer, is generally clear or pale yellow in color. The second layer is a narrow grayish white interface band representing the “buffy coat” or leukocyte fraction. The third or bottom layer is dark red and consists of the erythrocytes or red blood cells. Using an appropriate disposable transfer pipette, the plasma layer is aspirated off down to approximately 1 mm from the buffy coat layer taking care not to disturb the leukocyte or buffy coat layer. All plasma is expelled from the pipette into a plasma collection tubes. Recovered plasma is aliquoted and placed into labeled cryovials. The barcoded cryovials are placed in appropriate storage units for long-term storage in -80 °C freezers at the EM Papper laboratory at Nova Southeastern University.

3.3.1.2. Recovery of white blood cells. After removing the plasma layer, removal of buffy coat is performed. To isolate PBMCs, sufficient quantity of PBS is added to bring blood back to its original whole blood volume and mixed gently to continue PBMC processing. A transfer pipette is used to transfer all of the blood into a 15 mL tube containing 3 mL Ficoll-Hypaque solution. The tube is centrifuged (without a brake) at 2250 x g for 25 min at 25 °C. Using a sterile serological or transfer pipet, all cells are collected at the cloudy white interface taking care not to aspirate any more separation medium solution than necessary. The collected cells are transferred from one conical centrifuge tube to a single corresponding, pre-labeled, sterile conical centrifuge tube. After centrifugation of the wash step, cells are resuspended in 10 mL of PBS for cell count and viability using the Beckman Coulter ViCell Counter. For this study, 5×10^6 cells/ml per vial are aliquoted in final freezing solution of 70% RPMI 1640 with 20% fetal bovine serum and 10% dimethyl sulfoxide (DMSO) as a cryoprotectant added. The cryovials are placed in the appropriate storage units at -80 °C for short-term storage. For long-term storage, cells stored in freezing vials are transferred into the liquid nitrogen cryopreservation tanks and their location is mapped and recorded.

3.3.1.3. Separation of serum from blood samples. Blood is drawn into BD Vacutainer® SST™ Venous blood collection tube with separator gel. This tube is spun down prior to shipping to the EM Papper laboratory. Serum sample above the gel separator is collected and stored in barcoded cryovials at various aliquot volumes at -80 °C for long term storage.

Quality Assurance and Assessment protocols take place before, during and after the samples are isolated from primary tubes and placed into the biorepository. Samples for all four prospective resource sites are processed with the same protocols.

3.3.2. Common issues of aliquot size

Biorepositories face a lack of predictability in future research direction, limited resources in space and maintenance manpower, as well as new technology innovations, which makes planning a biorepository difficult. It is imperative to create a versatile sample aliquot scheme in order to combat these challenges. However, repositories commonly will need to be flexible with remaining sample subsets should circumstances change, while avoiding “freeze thaw cycles” that are potentially damaging to certain proteins in serum or plasma. While large aliquots of 2 mL

or greater can reduce maintenance costs and space requirements, small aliquots are the versatile option that allow for greater flexibility when fulfilling sample requests. This laboratory takes a different approach, setting up large numbers of small aliquots (0.5 and 0.25 mL) while still creating a small number of large aliquots (1.0 mL) for longer term storage (Fig. 4; Table 1).

5) Home specimen and data collection

Home collection by study participants includes urine, stool samples, and Fitbit data collection. Specific methods and details are listed below.

3.3.2.1. Fitbit data. Each participant is asked to wear a study-provided Fitbit activity monitor for 7 consecutive days. On the eighth day, participants are expected to extract their sleep quality and heart rate variability data and upload the results to the network coordinating center through a secure link. An instructional manual is provided to the participant for proper data extraction. All Fitbit data are made available for sharing in the repository.

3.3.2.2. Urine samples. Urine sample kits are provided to the participant with comprehensible instructions during the in-person study visit to complete at home and mail back in prepared pre-paid packaging. Participants are expected to collect at least 20 mL urine during their first morning void. Urine samples from all study sites are overnight shipped with an ice pack to maintain cold chain transport to the Boston University Medical Campus for aliquoting and storage. Once received, the urine samples are aliquoted, frozen and stored in the repository for later urinalysis, sharing and sample requests (Table 1).

3.3.2.3. Stool samples. Stool sample kits are provided to the participant during the in-person visit with clear instructions to complete the at home stool sample kit and mail it back in a prepared pre-paid packaging. Participants are expected to collect two tubes of stool. Stool samples from all study sites are overnight shipped to Boston University Medical Center for storage. Samples are stable for 15 days at room temperature and very well preserved over a long period of time at —4C. Samples will be stored at the Boston University Medical Center Laboratory and available for collaborative research and for requests through the repository.

4. Data sharing

The BBRAIN biorepository has a responsibility to provide access to samples for pilot work and other initiatives to further the field. This is accomplished by a three-stage approval process for any data or samples that exist as part of this project and/or the biorepository. This process is streamlined through a web-based data request platform created by the network coordinating center. The first steps involve a potential investigator contacting the project PI to determine validity of an idea. Once initial conversations are considered positive, the potential investigator submits a proposal, along with a sample or data request form, to the Steering Committee. This committee then assesses the proposal for feasibility (samples must be available, technology/assays must have a reasonable chance of success), duplicative effort (avoids overlap from funded portion of the grant or previously approved requests), and sample volume requested (protects the integrity of the aliquot scheme, i.e. requesting 1 mL when 200uL would suffice). Finally, the request is reviewed by the Steering Committee for further consideration and final approval or denial. There is no cost for the sharing of samples from the repository except the cost of shipping the samples to the approved requestors.

4.1. How to request samples and data

As previously described, the BBRAIN Repository Network provides samples and data from newly collected whole blood, RNA, DNA, plasma, serum, saliva, stool, and urine samples for 500 GW veterans (300 GWI cases, 200 controls) in addition to demographic surveys and cognitive test data. In addition, a repository of previously collected demographic and health survey, clinical (cognitive testing, MRI data) and preclinical data (animal tissue) has been compiled from the 15 participating GWI investigators and made available to the BBRAIN repository for data mining and sharing. 9 lines of hiPSCs collected from 5 GW-veterans with GWI and 4 from those who did not develop GWI are available upon request. As previously outlined, the Network Coordinating Center organizes approvals with the steering committee members in consultation with the biorepository contributor sites. The site for requesting prospective and retrospectives biospecimens, brain imaging and other health symptom or cognitive data is available at https://wwwapp.bumc.bu.edu/BEDAC_BBrainRetro.

5. Discussion

The BBRAIN is the first repository network designed to gather and store samples, provide new data and to mine data from prior studies of difficult to obtain samples (CSF, PET, MRI imaging). In doing so, BBRAIN has grown, and continues to shape and fill the need for an easily accessible biospecimen repository in the field of GWI research. An additional primary objective of this biorepository network has been to determine minimal data elements using a common data platform from retrospective studies, thus creating centralized resource websites for BBRAIN researchers and other interested GWI researchers seeking to obtain repository samples and data for analyses. The task has been completed, with common data elements for symptom and system domains now identified. To date, BBRAIN has published recommended common data elements for neuropsychological and other outcomes [15,47]. Importantly, the BBRAIN prospective sample and data collection is consistent with the same elements of the common data recommendations and will therefore provide additional validation of nearly all of the common data elements.

BBRAIN will build upon the initial progress of the Boston GWIC biorepository. It has been stated that the success of a biorepository is not in how many samples are collected but in how many samples are

Table 1
BBRAIN blood and saliva aliquot and storage procedures.

Sample type	Tube type/color	# tubes	Tube volume (ml)	Total volume (ml)	Product	Expected product volume - biobank	Aliquot scheme	Test
Blood draw	(3) Green Na Heparin	3	10	30	Blood, PBMC, Plasma	60 × 10^6 PBMC; 12 mL Plasma	PL2: 4 × 1 mL, 4× .5 mL, 24× .25 mL; CEL: 12 × 5 × 10^6/mL	NKCC, NPY, Nanostring
	(2) Red Tiger Top SST Tube	1	8	16	Serum	3 mL	2 × 0.5 mL; 8 × 0.25 mL	Hormone specific TBD
	(2) Purple EDTA	2	10	20	Blood, Serum	6 mL	4× .5 mL; 16× .25 mL	CBC, Flow, Cytokines
Saliva	(1) OGR-500	1	2	2	Saliva	2 mL	N/A	SNP, TBD
	(4) Salimetrics' Cryovials	4	2	8	Saliva	8 mL	2 × 1 mL; 12× .5 mL	Salivary Cortisol, TBD

shared that lead to important new results [55–58]. To date, BBRAIN has shared samples with five research investigators including cerebrospinal fluid, serum and plasma samples, PET brain imaging, diffusion MRI brain imaging data and cognitive outcomes [59]. Building on this strong foundation, BBRAIN aims to provide the infrastructure, scientific expertise, biological specimens and collaborative nature to vastly speed up objective biomarker discovery and treatments for ailing veterans with GWI. The robust infrastructure of the BBRAIN repository network will serve as a key resource for the GWI research field.

6. Conclusion

BBRAIN, built from a strong foundation of collaboration and need for a biorepository in the community of GWI research, aspires to provide the scientific resources to identify more definitive biomarkers for GWI. The team approach of sharing samples will lead to faster identification of diagnostic tests for GWI and targeted personalized medicine treatments for ill veterans. For veterans who have remained ill for over 30 years, the importance of quickly identifying diagnostic tests and effective treatments for GWI cannot be overstated.

Acknowledgements

The views expressed are those of the authors and do not necessarily reflect the official policy or position of the Department of Defense, the Department of Veterans Affairs, or the U.S. Government. This work was supported by the Office of the Assistant Secretary for Health, through the Gulf War Illness Research Program under Award No. W81XWH-18-1-0549 (Sullivan PI). We would like to thank all the Gulf War veterans who have agreed to share their samples for this biorepository.

References

[1] R.F. White, L. Steele, J.P. O'Callaghan, et al., 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 (2016) 449–475.
[2] RACGWI, Gulf War Illness and the Health of Gulf War Veterans: Research Cecommendations, US Government Printing Office, Washington, DC, 2008.
[3] A.L. Maule, P.A. Janulewicz, K.A. Sullivan, et al., Meta-analysis of self-reported health symptoms in 1990–1991 gulf war and gulf war-era veterans, BMJ Open 8 (2018) e016086.
[4] L. Steele, 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 (2000) 992–1002.
[5] K. Fukuda, R. Nisenbaum, G. Stewart, et al., Chronic multisymptom illness affecting air force veterans of the Gulf war, JAMA 280 (1998) 981–988.
[6] M.B. Abou-Donia, M.H. Krengel, E.S. Lapadula, et al., Sex-based differences in plasma autoantibodies to central nervous system proteins in gulf war veterans versus healthy and symptomatic controls, Brain Sci. 11 (2021).
[7] C.H. Cheng, B.B. Koo, S. Calderazzo, et al., Alterations in high-order diffusion imaging in veterans with gulf war illness is associated with chemical weapons exposure and mild traumatic brain injury, Brain Behav. Immun. 89 (2020) 281–290.
[8] G.J. Johnson, B.C. Slater, L.A. Leis, T.S. Rector, R.R. Bach, Blood biomarkers of chronic inflammation in gulf war illness, PLoS One 11 (2016) e0157855.
[9] M.B. Abou-Donia, E.S. Lapadula, M. Krengel, et al., Using plasma autoantibodies of central nervous system proteins to distinguish veterans with gulf war illness from healthy and symptomatic controls, Brain Sci. 10 (2020) 1–15.
[10] Y. Guan, C.H. Cheng, W. Chen, et al., Neuroimaging markers for studying gulf-war illness: single-subject level analytical method based on Machien learning, Brain Sci. 10 (2020) 1–13.
[11] Z. Alshelh, D.S. Albrecht, C. Bergan, et al., In-vivo imaging of neuroinflammation in veterans with gulf war illness, Brain Behav. Immun. 87 (2020) 498–507.
[12] 2012 best practices for repositories collection, storage, retrieval, and distribution of biological materials for research international society for biological and environmental repositories, Biopreserv. Biobank 10 (2012) 79–161.
[13] M. Garcia, J. Downs, A. Russell, W. Wang, Impact of biobanks on research outcomes in rare diseases: a systematic review, Orphanet J. Rare Dis. 13 (2018) 202.
[14] A.K. Cheema, R.B. McNeil, T. Craddock, et al., Gulf War Illness clinical trials and interventions consortium (GWICTIC): a collaborative research infrastructure for intervention and implementation, Life Sci. 278 (2021) 119636.
[15] DE Cohen A common language for Gulf War Illness (GWI) research studies: GWI common data elements. Life Sci. accepted.
[16] NK Lea Steele Maxine Krengel Emily Quinn Rosemary Toomey Deborah M. Little Maria Abreu Kristina Aenlle Ronald Killiany Bang-Bon Koo Patricia A. Janulewicz Timothy Heeren Allison N. Clark Joy Ajama Joanna Cirillo Gerardo Buentello Vanesa Lerma Kimberly Sullivan Brain-immune interactions as the basis of Gulf War Illness: clinical assessment and deployment profile of 1990-91 Gulf War Veterans in the Gulf War Illness Consortium (GWIC) multisite case-control study Brain Sci. Submitted.
[17] R.K. Seth, R. Maqsood, A. Mondal, et al., Gut DNA virome diversity and its association with host bacteria regulate inflammatory phenotype and neuronal immunotoxicity in experimental gulf war illness, Viruses 11 (2019).
[18] U. Joshi, A. Pearson, J.E. Evans, et al., A permethrin metabolite is associated with adaptive immune responses in gulf war illness, Brain Behav. Immun. 81 (2019) 545–559.
[19] S. Janulewicz, Carlson, The gut-microbiome in Gulf War veterans: a preliminary report, Int. J. Environ. Res. Public Health 16 (2019) 3751.
[20] J. Belgrad, D.J. Dutta, S. Bromley-Coolidge, et al., Oligodendrocyte involvement in gulf war illness, Glia 67 (2019) 2107–2124.
[21] D. Kimono, S. Sarkar, M. Albadrani, et al., Dysbiosis-associated enteric glial cell immune-activation and redox imbalance modulate tight junction protein expression in gulf war illness pathology, Front. Physiol. 10 (2019) 1229.
[22] J.J. Latimer, A. Alhamed, S. Sveiven, et al., Preliminary evidence for a hormetic effect on DNA nucleotide excision repair in veterans with gulf war illness, Mil. Med. 185 (2019) e47–e52.
[23] L.T. Michalovicz, K.A. Kelly, S. Vashishtha, et al., Astrocyte-specific transcriptome analysis using the ALDH1L1 bacTRAP mouse reveals novel biomarkers of astrogliosis in response to neurotoxicity, J. Neurochem. 150 (2019) 420–440.
[24] J.P. O'Callaghan, D.B. Miller, Neuroinflammation disorders exacerbated by environmental stressors, Metabolism 100s (2019) 153951.
[25] L.T. Michalovicz, A.R. Locker, K.A. Kelly, et al., 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 (2019) 26–32.
[26] P. Janulewicz, M. Krengel, E. Quinn, et al., The multiple hit hypothesis for gulf war illness: self-reported chemical/biological weapons exposure and mild traumatic brain injury, Brain Sci. 8 (2018).
[27] K.A. Kelly, L.T. Michalovicz, J.V. Miller, V. Castranova, D.B. Miller, J.P. O'Callaghan, Prior exposure to corticosterone markedly enhances and prolongs the neuroinflammatory response to systemic challenge with LPS, PLoS ONE 13 (2018) e0190546.
[28] B.-B. Koo, L.T. Michalovicz, S. Calderazzo, et al., Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of gulf war illness, Brain Behav. Immun. 67 (2018) 42–46.
[29] U. Joshi, J.E. Evans, R. Joseph, et al., Oleoylethanolamide treatment reduces neurobehavioral deficits and brain pathology in a mouse model of gulf war illness, Sci. Rep. 8 (2018) 12921.
[30] R.K. Seth, D. Kimono, F. Alhasson, et al., Increased butyrate priming in the gut stalls microbiome associated-gastrointestinal inflammation and hepatic metabolic reprogramming in a mouse model of gulf war illness, Toxicol. Appl. Pharmacol. 350 (2018) 64–77.
[31] A.N. Rao, A. Patil, Z.D. Brodnik, et al., Pharmacologically increasing microtubule acetylation corrects stress-exacerbated effects of organophosphates on neurons, Traffic 18 (2017) 433–441.
[32] M.B. Abou-Donia, L.A. Conboy, E. Kokkotou, et al., Screening for novel central nervous system biomarkers in veterans with gulf war illness, Neurotoxicol. Teratol. 61 (2017) 36–46.
[33] T. Emmerich, Z. Zakirova, N. Klimas, et al., Phospholipid profiling of plasma from GW veterans and rodent models to identify potential biomarkers of gulf war illness, PLoS One 12 (2017) e0176634.
[34] R.D. Fields, D.J. Dutta, J. Belgrad, M. Robnett, Cholinergic signaling in myelination, Glia 65 (2017) 687–698.
[35] P.A. Janulewicz, M.H. Krengel, A. Maule, et al., Neuropsychological characteristics of gulf war illness: a meta-analysis, PLoS ONE 12 (2017) e0177121.
[36] A.R. Locker, L.T. Michalovicz, K.A. Kelly, J.V. Miller, D.B. Miller, J.P. O'Callaghan, Corticosterone primes the neuroinflammatory response to gulf war illness-relevant organophosphates independently of acetylcholinesterase inhibition, J. Neurochem. 142 (2017) 444–455.
[37] J.P. O'Callaghan, L.T. Michalovicz, K.A. Kelly, Supporting a neuroimmune basis of gulf war illness, EBioMedicine 13 (2016) 5–6.
[38] L. Qiang, A.N. Rao, G. Mostoslavsky, et al., Reprogramming cells from gulf war veterans into neurons to study gulf war illness, Neurology 88 (2017) 1968–1975.
[39] L. Abdullah, J.E. Evans, U. Joshi, et al., Translational potential of long-term decreases in mitochondrial lipids in a mouse model of gulf war illness, Toxicology 372 (2016) 22–33.
[40] D. Bose, A. Mondal, P. Saha, et al., TLR antagonism by sparstolonin B alters microbial signature and modulates gastrointestinal and neuronal inflammation in gulf war illness preclinical model, Brain Sci. 10 (2020) 532.
[41] D. Kimono, D. Bose, R.K. Seth, et al., Host akkermansia muciniphila abundance correlates with gulf war illness symptom persistence via NLRP3-mediated neuroinflammation and decreased brain-derived neurotrophic factor, Neurosci. Insights. 15 (2020), 2633105520942480.
[42] L.T. Michalovicz, K.A. Kelly, K. Sullivan, J.P. O'Callaghan, Acetylcholinesterase inhibitor exposures as an initiating factor in the development of gulf war illness, a chronic neuroimmune disorder in deployed veterans, Neuropharmacology 171 (2020) 108073.
[43] C. Garza-Lombó, M. Thang, H.J. Greve, et al., Circulating HMGB1 is elevated in veterans with gulf war illness and triggers the persistent pro-inflammatory microglia phenotype in male C57Bl/6J mice, Transl. Psychiatry 11 (2021) 390.
[44] L.L. Chao, L. Abadjian, J. Hlavin, D.J. Meyerhoff, M.W. Weiner, Effects of low-level sarin and cyclosarin exposure and gulf war illness on brain structure and function: a study at 4T, Neurotoxicology. 32 (2011) 814–822.

[45] L.L. Chao, J.C. Rothlind, V.A. Cardenas, D.J. Meyerhoff, M.W. Weiner, 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 (2010) 493–501.
[46] K. Sullivan, M. Krengel, S.P. Proctor, S. Devine, T. Heeren, R.F. White, Cognitive functioning in treatment-seeking gulf war veterans: pyridostigmine bromide use and PTSD, J. Psychopathol. Behav. Assess. 25 (2003) 95–103.
[47] M.G. Jeffrey, M. Krengel, J.L. Kibler, et al., Neuropsychological findings in gulf war illness: a review, Front. Psychol. 10 (2019) 2088.
[48] K. Sullivan, M. Krengel, W. Bradford, et al., Neuropsychological functioning in military pesticide applicators from the Gulf war: effects on information processing speed, attention and visual memory, Neurotoxicol. Teratol. 65 (2018) 1–13.
[49] D.J. Buysse, C.F. Reynolds, T.H. Monk, S.R. Berman, D.J. Kupfer, The Pittsburgh sleep quality index (PSQI): a new instrument for psychiatric research and practice, Psychiatry Res. 28 (1989) 193–213.
[50] A.M. Boonstra, H.R. Schiphorst Preuper, M. Reneman, J.B. Posthumus, R.E. Stewart, Reliability and validity of the visual analogue scale for disability in patients with chronic musculoskeletal pain, Int. J. Environ. Res. Public Health 31 (2008) 165–169.
[51] L.E. Kazis, N. Wilson, Health Status of Veterans: Physical and Mental Component Summary Scores (SF-36V): 1996 National Survey of Ambulatory Care Patients, Office of Performance and Quality, and Health Assessment Project, Health Services Research and Development Service, Washington DC, and Bedford, MA, 1997, Mar, 1997 Executive Report.
[52] D.M. McNair, M. Lorr, L.F. Droppleman, Manual for the Profile of Mood States, Educational and Industrial Testing Services, San Diego, CA, 1971.
[53] S.P. Proctor, Development of a Strucutred Neurotoxicant Assessment Checklist (SNAC) for Clinical Use in Veteran Populations, Department of Veterans Affairs, VA Boston Healthcare System, 2006.
[54] E.M. Smets, B. Garssen, B. Bonke, J.C. De Haes, The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue, J. Psychosom. Res. 39 (1995) 315–325.
[55] National Biospeciment Network Blueprint, in: A. Friede, R. Crossman, R. Hunt, R.M. Li, S. Stern (Eds.), Constella Group, Inc., Durham, NC, 2003.
[56] Human Tissue and Biological Samples for Use in Research: Operational and Ethical Guidelines.
[57] World Medical Association Declaration of Helsinki, Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization;79: 373-374.
[58] National Bioethics Advisory Commission, Research Involving Human Biological Materials: Ethical Issues and Policy Guidance. August 1991 Vol I: Report and Recommendations fo the National Bioethics Advisory Committee, 1991.
[59] Chia-Hsin Cheng Zeynab Alshelh Yi Guan Kimberly A Sullivan L Marco B-BK Loggia Association of the tissue microstructural diffusivity and translocator protein PET in Gulf War Illness Brain Behav. Immun. Submitted.

Association of the tissue microstructural diffusivity and translocator protein PET in Gulf War Illness

Chia-Hsin Cheng[a], Zeynab Alshelh[b], Yi Guan[a], Marco L. Loggia[b], Kimberly Sullivan[c], Bang-Bon Koo[a*]

[a] *School of Medicine, Boston University, Boston, MA, USA*

[b] *Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, USA*

[c] *Department of Environmental Health, School of Public Health, Boston University, Boston MA USA*

* Corresponding Author

Abstract

About a third of all United States veterans who served in the 1991 Gulf War (GW) report a range of chronic health symptoms including fatigue, neurocognitive symptoms, and musculoskeletal pain. There is growing evidence supporting the detrimental effects of maladaptive neuroimmune reactions in this multi-symptom illness. Indeed, recent studies using positron emission tomography (PET) using the radioligand [^{11}C]PBR28, which binds the neuroinflammation marker 18 kDa translocator protein (TSPO), and diffusion magnetic resonance imaging (dMRI) have independently identified the anterior cingulate (ACC) and midcingulate cortices (MCC) as key regions for differentiating veterans with GWI from healthy controls (HC). Here, we used integrated (i.e., simultaneous) PET/MRI imaging techniques, paired with dMRI processing methods (neurite density imaging, NDI, and free-water diffusion tensor model to single-shell high-order dMRI), to directly evaluate the relationship between ACC and MCC microstructural tissue parameters, TSPO signal and clinical parameters in the same cohorts of 10 veterans with GWI and 19 HCs.

Within the regions evaluated, TSPO signal elevations were associated with restricted diffusivity in the extracellular compartment, while clinical measures were best explained by neurite density and cellular structure complexity measures. Our study is the first to provide evidence of a relationship between PET and dMRI modalities in GWI and suggests that microstructural changes in the ACC and MCC are correlated to mood symptoms and cognitive performances in GWI veterans.

1. Introduction

Approximately one third of the 700,000 U.S. veterans who served in the 1991 Gulf War (GW) experience an array of chronic health symptoms, characterized by fatigue and sleep problems, pain, neurological, cognitive, and mood symptoms, respiratory complaints, gastrointestinal problems, and skin symptoms, collectively termed Gulf War Illness (GWI) (White et al., 2016). Effects of organophosphate (OP) neurotoxicant exposures, such as pesticides, and sarin/cyclosarin nerve agents that were present in the GW theater, possibly coupled with heightened innate immune responses, may give rise to chronic symptom complaints experienced by veterans with GWI (Chao et al., 2011; Steele et al., 2012; Sullivan et al., 2018). Findings from animal studies suggest that the exposure to neurotoxicants, stress, and combat-related injuries to the central nervous system (CNS) may induce long-lasting neuroinflammatory

responses in GWI, characterized by dysregulated glial cell activation (Lacagnina et al., 2021; Macht et al., 2019; O'Callaghan et al., 2015). While evidence for neuroinflammation in GWI has until recently been mostly limited to the preclinical literature, in a recent positron emission tomography (PET) study with [^{11}C]PBR28, a second-generation radioligand for the 18kDa translocator protein (TSPO), our group observed widespread cortical elevations in neuroinflammation. Elevated [^{11}C]PBR28 signal was evident in regions including the anterior and midcingulate cortices (ACC and MCC, respectively), in veterans with GWI compared to healthy controls or healthy GW veterans (Alshelh et al., 2020). These abnormal neuro-immune responses appear to trigger downstream macro- and microstructural changes in the brain (Koo et al., 2018). Indeed, diffusion magnetic resonance imaging (dMRI) studies found that microstructural alterations (including the ACC) were associated with elevated levels of peripheral proinflammatory cytokines, and with worse fatigue symptoms, in veterans with GWI (Cheng et al., 2020).

Despite this converging evidence from separate studies, the relationship between PET neuroinflammatory signals and dMRI measures of microstructural alterations in GWI has never been directly explored in the same individuals.

In this study, we used integrated (i.e., simultaneous) [^{11}C]PBR28 PET/MRI to address this aim. Specifically, we sought to assess if changes in microstructural integrity were related to increased neuroinflammatory signaling in the same brain areas and tissue compartments. We applied two dMRI processing models, neurite density imaging (NDI) and free-water diffusion tensor model (FW-DTI) (Pasternak et al., 2014; Zhang et al., 2012) to single-shell high-order dMRI data collected in veterans with GWI (and a healthy control group) from a recent GWI PET study (Alshelh et al., 2020). Furthermore, we compared subjective clinical outcomes with multi-compartment diffusion measures to investigate the relationships between objective brain imaging markers and self-reported health symptoms veterans with GWI.

2. Materials and Methods

2.1. Participants

Brain imaging outcomes from 10 veterans with GWI (2 females, 49.6 ± 3.1 years old [mean ± SD]) and 19 healthy controls (HC, 11 females, 44.1 ± 13.04 years old) were obtained from an established GWI biorepository and were included for the analysis. GWI case status was defined by the Kansas GWI case criteria, which requires multiple or moderate-to-severe chronic symptoms reported in at least three out of six symptom domains (fatigue, somatic pain, neurological cognitive, gastrointestinal, respiratory, and skin abnormalities) (Steele, 2000). Clinical outcomes including self-reported scales of pain, mood and cognitive testing of sustained attention including the Short-Form McGill Pain Questionnaire (SF-MPQ2), Patient-Reported Outcomes Measurement Information System (Promis-29), Conner's Continuous Performance Test III (CPT3), and -because GWI shares some of the core symptoms reported by patient suffering from fibromyalgia- the 2011 American College of Rheumatology (ACR) Fibromyalgia Diagnostic Criteria survey were also obtained from the repository for analysis (Cella et al., 2010; Conners et al., 2000; Dworkin et al., 2009; Wolfe et al. 2011).

2.2. Procedures

We processed the single-shell, high-order dMRI data (b-value = 3000 s/mm^2, 60 gradient directions, TR/TE/voxel size: 119 msec/8000 msec/2.5×2.5×3 mm) acquired in the GWI PET

study by Alshelh et al. (2020) and shared through the Boston Biorepository and Integrative Network (BBRAIN) for GWI (Keating et al., submitted). Microstructural diffusion indices reporting neurite density (ND), orientation dispersion (OD), and isotropic diffusion (IsoVF) were reconstructed using the NDI model in a similar protocol as described previously (Cheng et al., 2020). FW-DTI derived measures, free-water corrected fractional anisotropy (FW-FA), and free-water corrected mean diffusivity (FW-MD), were extracted using the single shell free-water elimination diffusion tensor model (Pasternak et al., 2014). Reconstructed diffusion maps from NDI and FW-DTI models were then linearly registered to the Montreal Neurological Institute 152 (MNI152) structural template and projected to hemispheric cortical surfaces (Greve et al., 2014). For the PET analysis, standardized uptake value ratio (SUVR) images were generated from data collected over the 60-90 min post-injection [^{11}C]PBR28 PET interval, preprocessed and transformed to MNI152 space, as previously described (Alshelh et al. 2020). The two regions of interest (ROI), ACC and MCC, were structures that displayed significantly elevated [^{11}C]PBR28 PET signal and significant microstructural alterations in GWI veterans compared to healthy controls in previous studies (Alshelh et al., 2020, Cheng et al., 2020). Both ROIs were projected to the standard space cortical surfaces using the same method as the reconstructed dMRI maps. Mean dMRI measures and SUVR values were then extracted from these regions. Clinical variables were also obtained as described by Alshelh et al. (2020).

Partial correlations were performed to assess the relationship between dMRI measures and (1) [^{11}C]PBR28 signal (controlling for sex, age, and TSPO genotype polymorphism, which predicts binding affinity to the PET radioligand) (Owen et al., 2012) and (2) clinical variables (controlling for sex and age). Significant p-values ($p < 0.05$) were reported along with Spearman correlation coefficients (rho).

3. Results

In the whole group (including GWI veterans and HC) analysis, elevated [^{11}C]PBR28 PET signal was significantly correlated with lowered IsoVF in the MCC (rho = -0.498, p = 0.008) and lowered FW-MD in the ACC (rho = -0.382, p = 0.049). The same patterns (MCC IsoVF: rho = -0.801, p = 0.017; ACC FW-MD: rho = -0.818, p = 0.013) were found in the analysis performed within veterans with GWI (Fig. 1). Other multi-compartment diffusion measures did not show significant patterns to [^{11}C]PBR28 PET signals in either ROI.

The results of the partial correlation analyses between clinical variables and multi-compartment diffusion measures in GWI veterans are listed in Table 1. Positive correlations were evident between (1) CPT3 scores and OD in both ACC (detectability p = 0.043, omission errors p = 0.018, variability p = 0.048) and MCC (detectability p = 0.032, omission errors p = 0.043, commission errors p = 0.046), (2) CPT3 HRT block change test and ND in the ACC (p = 0.044), (3) SFMPQ-2 sensory sum and ND in the MCC (p = 0.027), and (4) ACR total score and OD in the ACC (p = 0.016). Negatively correlations were found between (1) Promis-29 anxiety domain scores and IsoVF in the ACC (p = 0.042), (2) CPT3 scores and ND in the MCC (detectability p = 0.03, omissions p = 0.022), and (3) CPT3 HRT and OD in the MCC (p = 0.05).

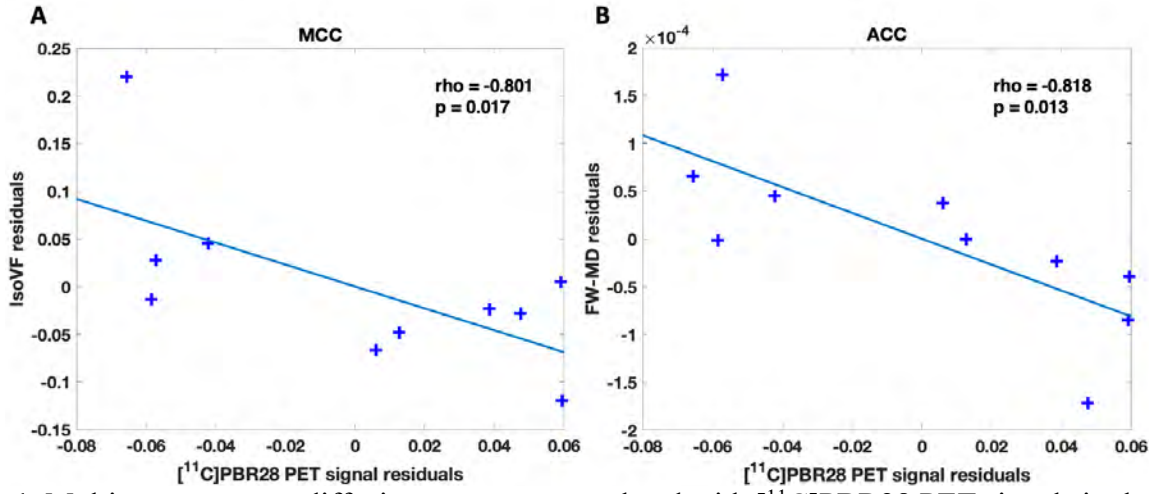


Fig. 1. Multi-compartment diffusion measures correlated with [^{11}C]PBR28 PET signals in the ACC and MCC in GWI veterans. Scatter plots illustrate the negative correlations between the neuroinflammation marker TSPO ([^{11}C]PBR28 PET signal) and (A) the isotropic diffusion measure (IsoVF) in the MCC, or with (B) the free-water corrected mean diffusivity (FW-MD) in the ACC. Results are plotted using TSPO genotype-, sex-, and age-controlled [^{11}C]PBR28 PET signal and age- and sex-controlled diffusion measures. Partial correlation coefficients (rho) are shown in the plots along with p-values. MCC = midcingulate cortex; ACC = anterior cingulate cortex.

Table 1. GWI multi-compartment diffusion measures correlations with clinical variables

	ACC			MCC	
	ND	IsoVF	OD	ND	OD
Promis29 Raw score: Anxiety	0.216	**-0.724***	-0.416	0.601	-0.674
SF-MPQ2: Sensory Sum	-0.369	-0.604	-0.111	**0.765***	-0.532
Conner's CPT3: Detectability	-0.345	0.593	**0.723***	**-0.755***	**0.751***
Conner's CPT3: Omissions	-0.040	0.647	**0.797***	**-0.780***	**0.722***
Conner's CPT3: Commissions	-0.371	0.525	0.501	-0.656	**0.716***
Conner's CPT3: Hit Reaction Time (HRT)	0.272	-0.502	-0.198	0.466	**-0.707***
Conner's CPT3: Variability	-0.551	0.423	**0.711***	-0.455	0.510
Conner's CPT3: HRT Block Change	**0.720***	0.202	-0.327	-0.191	0.174
ACR Total score	-0.146	0.623	**0.806***	-0.469	0.354

Partial correlation coefficients are reported, significant ($p<0.05$) results are bolded. * $p < 0.05$.

4. Discussion

Our study revealed significant relationships between the upregulation of the neuroinflammatory marker TSPO, detected by [^{11}C]PBR28 PET signals, and both decreased extracellular isotropic diffusivity, captured by IsoVF, and lowered cellular packing density, measured by FW-MD, in the ACC and MCC of GWI veterans. This study is an extension to our previous analyses, which showed elevated levels of the neuroinflammatory marker TSPO in a widespread set of brain regions in GWI (Alshelh et al., 2020). The neuroinflammatory marker TSPO is normally expressed at low levels but becomes dramatically upregulated -predominantly in activated glial cells- during neuroinflammatory responses (Rupprecht et al., 2010). TSPO

signal elevations observed in diseases such as fibromyalgia and GWI (Albrecht et al., 2019; Alshelh et al., 2020) suggest that dysregulated glial activation may contribute to the pathophysiology of GWI, as suggested by the preclinical literature. The present study is the first to report dMRI correlates of GWI neuroinflammation. The negative correlations between IsoVF and [^{11}C]PBR28 PET signal in the MCC suggested hindered isotropic diffusivity, which could arise from local glial activation or immune cell infiltration (Yi et al., 2019; Zhang et al., 2012). In fact, decreased IsoVF was shown to reflect chronic stages of microglia-mediate neuroinflammation, which corroborate findings from previous GWI PET studies (Alshelh et al., 2020; Yi et al., 2019). We also observed a similar pattern between elevated [^{11}C]PBR28 PET signal and lowered FW-MD in the ACC, which suggested decreased cellular packing density that could be reflecting gliosis or cytoarchitecture disruption (Pasternak et al., 2014). In recent GW studies, biomarkers indicating the presence of neuronal injury and gliosis were detected in veterans with GWI 30 years after the war, and in GW rat models, the disruption of oligodendrocyte development and changes in glial morphology were identified as key components in studying the chronic neuroinflammatory responses in GWI (Abou-donia et al., 2020; Belgrad et al., 2019). Indeed, our findings on the relationship between [^{11}C]PBR28 signal and dMRI measures confirmed that neuroinflammatory responses may be accompanied by microstructural diffusion alterations in GWI.

Interestingly, while our prior PET analyses didn't reveal statistically significant association with clinical variables, our multi-compartment dMRI measures extracted from the same ACC/MCC regions were correlated with measures of pain, anxiety, and cognitive performance, all symptom domains commonly affected in veterans with GWI (Janulewicz et al., 2017; Jeffrey et al., 2019; Sullivan et al., 2018; White et al., 2016). Our results showed that lower IsoVF and OD in the ACC were associated with worse anxiety, while higher ND in the MCC was related to higher pain severity.

Veterans with GWI often report sensorimotor deficits and memory impairments, often coupled with heightened innate immune responses that give rise to chronic symptom complaints (Chao et al., 2011; Janulewicz et al., 2017; Jeffrey et al., 2019; Sullivan et al., 2018). Furthermore, animal models of GWI show that stress combined with exposure to chemicals present in the GW theater (pesticides, and sarin/cyclosarin nerve agents) produces GWI symptoms greater than exposure to chemicals alone and causes neuronal cell death in the cingulate cortex (O'Callaghan and Miller, 2019). These changes in the cingulate cortex cause neurobehavioral changes similar to those observed in GWI veterans (Abdullah et al., 2012; Macht et al., 2019). In our study, higher rates of CPT3 detectability, omission and commission errors, and response variabilities were found to correlate with higher OD and lower ND in veterans with GWI, whereas slower and varying response rates were associated with lower OD and higher ND. Similar results were reported in other studies, which documenting an association between reduced gray and white matter volumes and higher rates of CPT3 omission errors in GW veterans exposed to chemical warfare agents (Chao et al., 2011). Lower ND accompanied with higher OD could reflect decreases in overall volume on the microscopic level and increases in diffusion tortuosity (Colgan et al., 2016), while higher ND has been linked to less efficient information processing capacity (Genç et al., 2018). However, our ability to the precisely interpret the multi-compartment dMRI measures is limited, particularly as the relationship between microstructural alterations and dMRI measures can vary depending on the etiology of the disease.

In the current study, we demonstrated the presence of changes in the microstructural environments of the ACC and MCC, associated neuroinflammatory signals and behavioral measures, in a small GWI sample. Future studies with a larger GW veteran cohort and post-mortem human studies will be required to shine more light on the neurobiological correlates of neuroinflammation in GWI.

Acknowledgments

This work is supported in part by DoD-W81XWH1810549 (KS), DoD-W81XWH-17-1-0440 (BBK), DoD-W81XWH-14-1-0543 (MLL), R01-NS094306-01A1 (MLL), and R01-NS095937-01A1 (MLL).

References

Abdullah, L., Evans, J.E., Bishop, A. Reed, J.M., Crynen, G., Phillips, J., Pelot, R., Mullan, M.A., Ferro, A., Mullan, C.M. 2012. Lipidomic Profiling of Phosphocholine Containing Brain Lipids in Mice with Sensorimotor Deficits and Anxiety-Like Features After Exposure to Gulf War Agents. Neuromol. Med. 14, 349-361.

Abou-Donia, M.B., Lapadula, E.S., Krengel, M.H., Quinn, E., LeClair, J., Massaro, J., Conboy, L.A., Kokkotou, E., Abreu, M., Klimas, N.G., Nguyen, D.D., Sullivan, K. 2020. Using Plasma Autoantibodies of Central Nervous System Proteins to Distinguish Veterans with Gulf War Illness from Healthy and Symptomatic Controls. Brain Sci. 10(9), 610.

Albrecht, D.S., Forsberg, A., Sandstrom, A., Bergan, C., Kadetoff, D., Protsenko, E., Lampa, J., Lee, Y.C., Hoglund, C.O., Catana, C., et al., 2019. Brain glial activation in fibromyalgia - A multi-site positron emission tomography investigation. Brain Behav. Immun. 75, 72–83.

Alshelh, Z., Albrecht, D.S., Bergan, C., Akeju, O., Clauw, D.J., Conboy, L., Edwards, R.R., Kim, M., Lee, Y.C., Protsenko, E., et al. 2020. In-vivo imaging of neuroinflammation in veterans with Gulf War illness. Brain Behav. Immun. 87, 498-507.

Belgrad, J., Dutta, D.J., Bromley-Coolidge, S., Kelly, K.A., Michalovicz, L.T., Sullivan, K.A., O'Callaghan, J.P., Fields, R.D. 2019. Oligodendrocyte involvement in Gulf War Illness. Glia 67(11), 2107-2124.

Cella, D., Riley, W., Stone, A., Rothrock, N., Reeve, B., Yount, S., Amtmann, D., Bode, R., Buysse, D.J., Choi, S.W., et al. 2010. The Patient–Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008. *Journal of Clinical Epidemiology* 63, 1179-1194.

Chao, L.L., Abadjian, L., Hlavin, J., Meyerhoff, D.J., Weiner, M.W. 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.

Cheng, C-H., Koo, B-B., Calderazzo, S., Quinn, E., Aenelle, K., Steele, L., Klimas, N., Krengel, M., Janulewicz, P., Toomey, R., et al. 2020. Alterations in high-order diffusion imaging in veterans with Gulf War Illness is associated with chemical weapons exposure and mild traumatic brain injury. Brain Behav. Immun. 89, 281-290.

Colgan, N., Siow, B., O'Callaghan, J.M., Harrison, I.F., Wells, J.A., Holmes, H.E.,

Ismail, O., Richardson, S., Alexander, D.C., Collins, E.C., et al. 2016. Application of neurite orientation dispersion and density imaging (NODDI) to a tau pathology model of Alzheimer's disease. NeuroImage 125, 739-744.

Conners, C.K., Staff, M., Connelly, V., Campbell, S., MacLean, M., Barnes, J. 2000. In: Conners' continuous performance Test II (CPT II v. 5). Multi-Health Syst Inc, 175–196.

Dworkin, R.H., Turk, D.C., Revicki, D.A., Harding, G., Coyne, K.S., Peirce-Sandner, S., Bhagwat, D., Everton, D., Burke, L.B., Cowan, P., et al. 2009. Development and initial validation of an expanded and revised version of the Short-form McGill Pain Questionnaire (SF-MPQ-2) *Pain* 144, 35–42.

Genç, E., Fraenz, C., Schlüter, C., Friedrich, P., Hossiep, R., Voelkle, M.C., Ling, J.M., Güntürkün, O., Jung, R.E. 2018. Diffusion markers of dendritic density and arborization in gray matter predict differences in intelligence. *Nat. Commun.* 9, 1905.

Greve, D.N., Svarer, C., Fisher, P.M., Feng, L., Hansen, A.E., Baare, W., Rosen, B., Fischl, B., Knudsen, G.M. 2014. Cortical surface-based analysis reduces bias and variance in kinetic modeling of brain PET data. Neuroimage 92, 225-236.

Janulewicz, P.A., Krengel, M.H., Maule, A., White, R.F., Cirillo, J., Sisson, E., Heeren, T., Sullivan, K. 2017. Neuropsychological characteristics of Gulf War illness: A meta-analysis. PLoS One. 12(5): e0177121.

Jeffrey, M.G., Krengel, M., Kibler, J.L., Zundel, C., Klimas, N.G., Sullivan, K., Craddock, T.J.A. 2019. Neuropsychological Findings in Gulf War Illness: A Review. Front Psychol. 10, 2088.

Koo, B.B., Michalovicz, L.T., Calderazzo, S., Kelly, K.A., Sullivan, K., Killiany, R.J., O'Callaghan, J.P. 2018. Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. Brain Behav. Immun. 67, 42–46.

Lacagnina M.J., Li, J., Lorca, S., Rice, K.C., Sullivan, K., O'Callaghan, J.P., Grace, P.M. 2021. A role for neuroimmune signaling in a rat model of Gulf War Illness-related pain. Brain Behav Immun. 91, 418-428.

Macht, V.A., Woodruff, J.L., Maissy, E.S., Grillo, C.A., Wilson, M.A., Fadel, J.R., Reagan, L.P. 2019. Pyridostigmine bromide and stress interact to impact immune function, cholinergic neurochemistry and behavior in a rat model of Gulf War Illness. Brain Behav. Immun. 80, 384-393.

O'Callaghan, J.P., Kelly, K.A., Locker, A.R., Miller, D.B., Lasley, S.M. 2015. Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of Gulf War Illness. J. Neurochem. 133, 708–721.

O'Callaghan, J.P., Miller, D.B., 2019. Neuroinflammation disorders exacerbated by environmental stressors. Metab. Clin. Exp. 100s, 153951.

Owen, D. R., Yeo, A. J., Gunn, R. N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D. J., Bennacef, I., Parker, C. A., et al. 2012. An 18-kDa Translocator Protein (TSPO) Polymorphism Explains Differences in Binding Affinity of the PET Radioligand PBR28. Journal of Cerebral Blood Flow & Metabolism *32*(1), 1–5.

Pasternak, O., Maier-Hein, K., Baumgartner, C., Shenton, M.E., Rathi, Y., Westin, C.F. 2014. The Estimation of Free-Water Corrected Diffusion Tensors. In: Westin CF., Vilanova A., Burgeth B. (eds) Visualization and Processing of Tensors and Higher Order

Descriptors for Multi-Valued Data. Mathematics and Visualization. Springer, Berlin, Heidelberg.

Rupprecht, R., Papadopoulos, V., Rammes, G., Baghai, T.C., Fan, J., Akula, N., Groyer, G., Adams, D., Schumacher, M. 2010. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat. Rev. Drug Discov. 9, 971–988.

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.

Steele, L., Sastre, A., Gerkovich, M.M., Cook, M.R. 2012. Complex factors in the etiology of Gulf War illness: wartime exposures and risk factors in veteran subgroups. Environ Health Perspect. 120(1), 112-118.

Sullivan, K., Krengel, M., Bradford, W., Stone, C., Thompson, T.A., Heeren, T., White, R.F. 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.

White, R.F., Steele, L., O'Callaghan, J.P., Sullivan, K., Binns, J.H., Golomb, B.A., Bloom, F.E., Bunker, J.A., Crawford, F., Graves, J.C., et al. 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.

Wolfe, F., Clauw, D.J., Fitzcharles, M.A., Goldenberg, D.L., Hauser, W., Katz, R.S., Mease, P., Russell, A.S., Russell, I.J., Winfield, J.B. 2011. Fibromyalgia criteria and severity scales for clinical and epidemiological studies: a modification of the ACR Preliminary Diagnostic Criteria for Fibromyalgia. J. Rheumatol. 38, 1113–1122.

Yi, S.Y., Barnett, B.R., Torres-Velázquez, M., Zhang, Y., Hurley, S.A., Rowley, P.A., Hernando, D., Yu, J.J. 2019. Detecting Microglial Density With Quantitative Multi-Compartment Diffusion MRI. Frontiers in neuroscience 13, 81.

Zhang, H., Schneider, T., Wheeler-Kingshott, C.A., Alexander, D.C. 2012. NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. NeuroImage 61, 1000–1016.

A comparison of different brain connectivity markers for classifying Gulf-war illness

Bang-Bon Koo and Kimberly Sullivan

Introduction

Symptoms of Gulf War illness (GWI) typically include persistent headaches, widespread pain, fatigue, memory and concentration problems and other difficulties in this complex multi-system disorder. Converging evidence suggests that a clear understanding of brain-immune interactions can help us to understand the origin of these symptoms. Morphometric neuroimaging analysis on Gulf War (GW) veterans exposed to neurotoxic and nerve agents confirmed overall reduction in the grey matter (GM)[1] and white matter (WM)[2,3], compared to the non exposed veterans. These regional changes in the morphometry of various brain regions have also been tied to changes in brain connectivity using diffusion MRI[4,5]. These findings may indicate that there are focal spots primarily involved in the illness propagation in the brain. In this study, we have applied a machine learning framework to diffusion magnetic resonance imaging markers from gulf war veterans to assess brain connections specific to Gulf-War illness (GWI).

Methods

Training set was based on 20 GW veterans (8 GW controls and 12 GWI, based on Kansas criteria) and 18 GW veterans' data was used for test set. We also included non-veteran aged control data (12 controls) in this study which have the same diffusion MRI data. Brain structural network was extracted from high angular resolution diffusion imaging (HARDI, spin-echo epi, 1.75/1.75/2mm voxel, 65 independent diffusion gradient directions, b-value 3000s/mm^2) data collected in the Boston Gulf War Illness Consortium (GWIC). Local Brain connection was defined based on the existence white matter tracts between each of the GM regions of interests (Freesufer ROIs, total 78). Per each local brain connections, we applied following quantifications for defining local connectivity measures: 1) total number of tracts, 2) tract mean value of microscale diffusivity[6] from generalized q-space reconstruction. We applied random decision forest classification for machine learning of the brain connectivity. Machine learning classifier was trained for different quantifications and compared each other. Performance of the machine learning classifier was tested based on a leave one out cross validation (LOOCV) and test set classification performance.

Results

The highest classification performance was confirmed in microscale diffusivity quantification. Classification based on the microscale diffusivity revealed accuracy of 84% in the LOOCV. In the test data validation, it showed 77% on classifying GWI. From the microscale diffusivity based classification, veterans with GWI had significant connectional alterations in several cortical regions compared to control veterans (Figure). Important connection features extracted by the classification model were found in the regions including 'the thalamus (L39) - frontal pole (L31)', 'the posterior cingulate (R22) - precuneous (R24)', 'the hippocampus (L37) - thalamus (L39)' and 'the insula (R34) - medial orbitofrontal (R13)' connections. Classification based on the total number of tracts revealed accuracy of 54% in the LOOCV. In the test data validation, it showed 46% on classifying GWI. Classifying between GWI and non-veteran aged control based on the total number of connections showed 90% in both LOOCV and test dataset classification.

Discussion

Brain connectomic techniques have potential power for uncovering the underlying mechanisms of GWI. The advantage of the brain connectomics lies in their capacity to map effects of interest in both focal as well as a large-scale data points. Brain connectomics allows for processing of a large and distributed number of brain connections as well as more local and focal connections. However, our preliminary data showed that different quantification strategies in diffusion MRI can have significant impact on describing

group characteristics. Lower range of diffusion encodings used in diffusion MRI (typically, around b=1000s/mm^2) is the most common set up in the clinical imaging and draws mostly the fast diffusion components and is useful for assessing WM major pathways, edemas or brain tumors. However, it might not have enough sensitivity to detect microscopic water diffusivity changes. Better sensitivity for detecting micro-diffusivity can be obtained by adding high diffusion strength encoding (typically, b>1,800s/mm^2) into the low diffusion setup. As shown in our preliminary observations, micro-diffusion can be a sensitive index to more diffuse changes in the brain.

Conclusion

Combining machine learning technology to brain connectivity imaging may allow for better understanding of the complex pathobiology of GWI. Choosing optimal imaging index should be a first step to maximize its classification performance. We are now extending our work by adding blood cytokine and cognitive measurements to the existing neuroimaging data to test multimodal data classification on GWI. We hope to present our data in the upcoming conference.

References

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

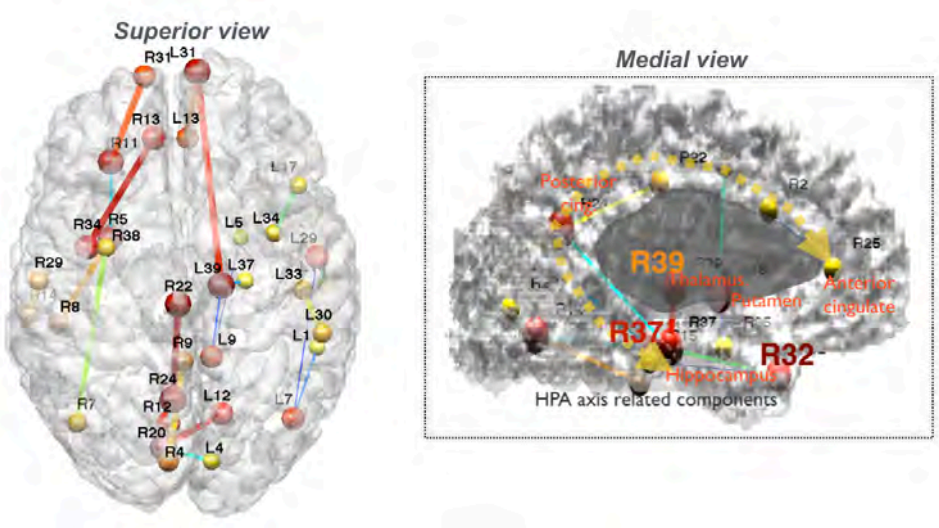
[2] Heaton KJ, Palumbo CL, Proctor SP, Killiany RJ, Yurgelun-Todd DA, White RF. Quantita- tive magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. Neurotoxicology 2007;28:761–9.

[3] Chao LL, Abadjian L, Hlavin J, Meyerhoff DJ, Weiner MW.Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: a study at 4 Tesla. Neurotoxicology 2011;32:814–22.

[4] Rayhan, R. U., Stevens, B. W., Timbol, C. R., Adewuyi, O., Walitt, B., VanMeter, J. W., et al. (2013). Increased brain white matter axial diffusivity associated with fatigue, pain and hyperalgesia in Gulf War illness [Research Support, N.I.H., Extramural, U.S. Gov't, Non-P.H.S.] PLoS One, 8(3), e58493.

[5] Chao, L. L., 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.

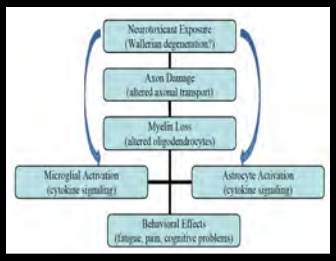
[6] Yeh, Fang-Cheng, Li Liu, T. Kevin Hitchens, and Yijen L. Wu, "Mapping Immune Cell Infiltration Using Restricted Diffusion MRI", *Magn Reson Med.* accepted, (2016)

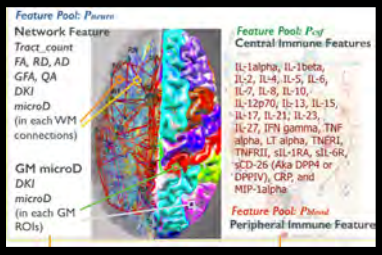


RESEARCH

Brain connectomic as a biomarker of Gulf War Illness

The central hypothesis for the pathobiological mechanisms of GWI in this consortium includes chronic neuroinflammation as a result of initial glial *activation* and then *priming* of glial responses that cause stronger and longer responses that do not shut off the chemical cascade of proinflammatory cytokines and chemokines that cross-talk between the immune system and the brain. This could result in a lasting multisystem illness affecting many body systems, as seen in GWI.





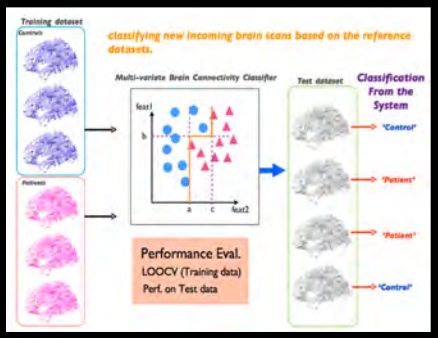
Identifying a reliable biomarker of Gulf-War Illness (GWI) has been a focus of the Boston Gulf War Illness Consortium (GWIC). Symptoms of GWI include fatigue, pain and cognitive problems. The GWIC is designed to compare these symptoms with proinflammatory cytokine and brain imaging biomarkers. Tools that assess the brain as a network have the potential to provide insight into how connectivity breaks down in response to chronic disease.

Courtesy: Bang-Bon Koo, Boston University

PHILIPS

RESEARCH

Brain connectomic as a biomarker of Gulf War Illness

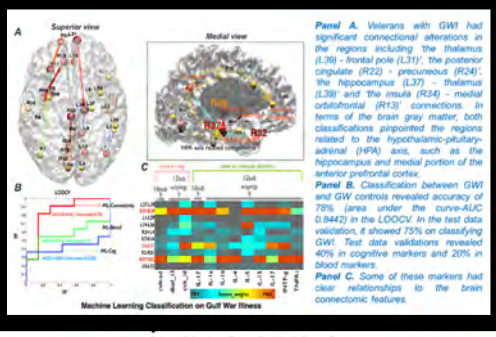


Courtesy: Bang-Bon Koo, Boston University

PHILIPS

RESEARCH

Brain connectomic as a biomarker of Gulf War Illness

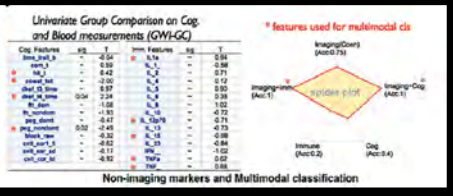


Panel A. *Veterans with GWI had significant connectional alterations in the regions including 'the thalamus (L39) - frontal pole (L31)', 'the posterior cingulate (R22) - precuneous (R24)', 'the hippocampus (L37) - thalamus (L39)' and 'the insula (R24) - medial orbitofrontal (R13)' connections. In terms of the brain gray matter, both classifications pinpointed the regions related to the hypothalamic-pituitary-adrenal (HPA) axis, such as the hippocampus and medial portion of the anterior prefrontal cortex.*

Panel B. *Classification between GWI and GW controls revealed accuracy of 78% (area under the curve-AUC 0.8442) in the LOOCV. In the test data validation, it showed 75% on classifying GWI. Test data validations revealed 40% in cognitive markers and 20% in blood markers.*

Panel C. *Some of these markers had clear relationships to the brain connectomic features.*

Combining some of either blood immune or cognitive markers with the neuroimaging markers (red asterisks in the left table) helped to improve the overall classification results on the test dataset (spider plot). Combining neuroimaging markers with either blood immune or cognitive measures revealed 100 % accuracy in classifying GWI in both classifiers.



Courtesy: Bang-Bon Koo, Boston University

PHILIPS

GWIC

GWIC Imaging

B. Koo
K. Sullivan
D. Little
BUMC, BCM

Spring 2018 - updat

1

GWIC

Imaging Items to discuss

Ultimate goal to devise an Objective Diagnostic Marker of GWI

Current Strategies for developing Diagnostic marker:

- Connectomics, Dr. Koo's multimodal imaging grant and progress to date
- Simple gray and white matter volumetric analyses
- Simple analyses of white matter pathways
- Complex analyses of white matter pathways
- Clinical impression of brain imaging outcomes – Dr. Little will discuss
- Inter-relationships to date of potential objective markers

2

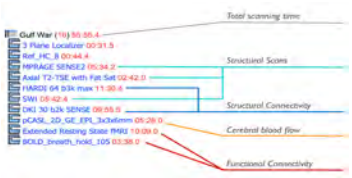
Multimodal Imaging in Gulf-war Illness

Bang-Bon Koo, Ph.D.

3

Multimodal Imaging in GWI

- Imaging protocol is designed to investigate both the individual and synergistic effects of structure, function, connectivity and blood flow of the brain secondary to Gulf war illness.



+ FDG+PET scans

59 Cases / 13 Controls

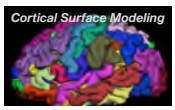
4 Cases / 3 Controls

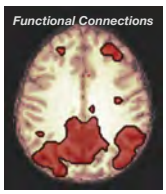
4

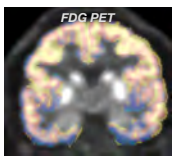
Multimodal Image Processing Pipelines

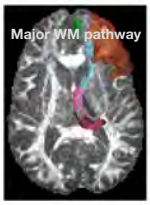
- **Aim I** Automated Image Processing Environment

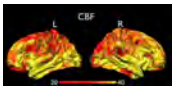
Applying current brain mapping technologies to the GWI imaging research









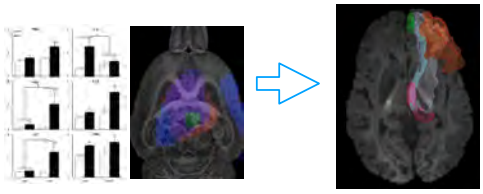


5

Multimodal Image Processing Pipelines

- **Aim II** Developing Novel Image Processing Scheme

Novel diffusion imaging technique (high-dimensional diffusion MRI) tested in GWI animal model has transferred to GWIC dataset

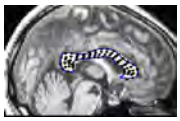


6

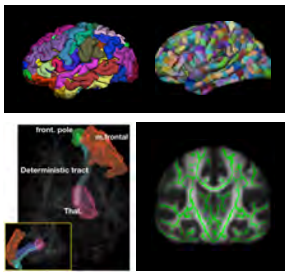
Multimodal Image Processing Pipelines

Aim II Developing Novel Image Processing Scheme

Novel Shape Deformity Assessment Algorithm on Subcortical Structures



Defining Features in Different Scales to find more sensitive markers



7

Multimodal Image Processing Pipelines

Aim III

Develop a novel supervised machine learning (ML) framework for the multi-modal biological dataset to establish a single subject level diagnostic inferences on GWI.

Building ML classifier for Imaging, Blood immune and Cognitive Data

Giving Statistical Inference

90%

70%

…

8

Multimodal Image Processing Pipelines

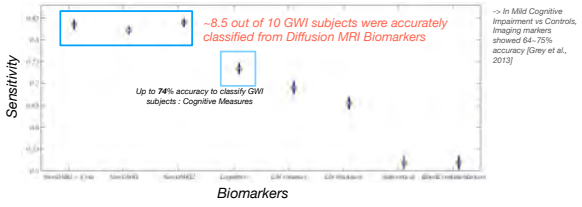
Aim III

Develop a novel supervised machine learning (ML) framework for the multi-modal biological dataset to establish a single subject level diagnostic inferences on GWI.

Building ML classifier for Imaging, Blood immune and Cognitive Data

Cross-Validations (38 GWcase vs 12 GWcon, 20 training set)

Diffusion MRI markers



-> In Mild Cognitive Impairment vs Controls, Imaging markers showed 64–75% accuracy (Grey et al., 2013)

9

Multimodal Image Processing Pipelines

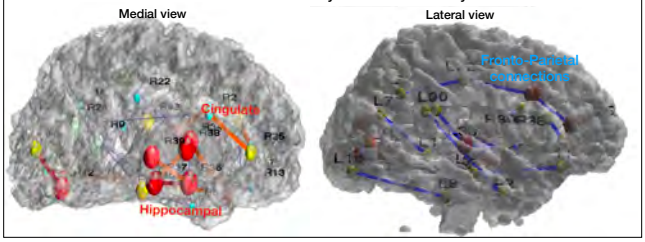
Aim III

Develop a novel supervised machine learning (ML) framework for the multi-modal biological dataset to establish a single subject level diagnostic inferences on GWI.

Building ML classifier for Imaging, Blood immune and Cognitive Data

Cross-Validations (38 GWcase vs 12 GWcon)

Diffusion MRI markers selected by the classification system



10

Multimodal Imaging in GWI

Next Steps

- More Subject Data (250) will be added to the Classifier
- Different Classification Schemes are being added into the system and compared each other to improve accuracy over 90%
  - Smart Datebase v1.0
- Longitudinal data acquisition will be planned along with BBRAIN - a great opportunity to add time component to the classification system to allow predicting future risks or treatment outcomes

11

BRAIN VOLUMES BY CASE STATUS N=72

Brain Area	GWI case Mean	Control Mean	P-value
Total Gray matter volume	595744	645131	0.01
Total cortex volume*	434566	452208	0.05
Precentral gyrus*	24465	27626	0.02
Caudal middle frontal gyrus	11595	13314	0.01
Pars-opercularis	7602	8207	0.05
Rostral middle frontal gyrus	19208	20989	0.03
Superior frontal gyrus	48851	52995	0.03
Pars-triangularis WM*	6766	6084	0.01
Superior Long. fasciculus WM* parietal endings -rh	1044	1271	0.005

*Significance in multivariate analyses after controlling for age, gender, ICV

12

Preliminary Evaluation of Diffusion Imaging Features for Classifying Veterans with Gulf War Illness

C. Cheng[1], D. Little[2], L. Steele[2], T. Heeren[3], R. Killiany[1], K.Sullivan[3], B. Koo[1]
[1]Anatomy and Neurobiology, Boston University School of Medicine; [2]Baylor College of Medicine;[3]Boston University School of Public Health

BOSTON UNIVERSITY
Anatomy & Neurobiology

Introduction

■ Gulf War Illness (GWI), is characterized by a combination of symptoms including widespread pain, fatigue and neuropsychological impairments.

- *These symptoms have been thought to be developed as a result of an innate immune response to a variety of different types of factors, such as toxic insults, injury or infection.*
- *Persisting symptoms of GWI has been shown to coincide with a heightened or chronic inflammatory reaction [1,2].*

■ Neuroimaging observations on GWI: evidences that the central nervous system is compromised in GWI has been found in studies using structural and functional magnetic resonance imaging (MRI).

- *Degenerative patterns: overall reduction in the grey matter (GM) and white matter (WM) in GW veterans exposed to neurotoxic agents / Reduced Hippocampal volume [3].*
- *Enhanced patterns: axial diffusivity (AD) measures in some WM major fiber pathways in GW veterans exposed to neurotoxic agents - not clear whether the observed patterns indicate WM reorganization [4].*
- *These complex findings require the use of a new brain mapping scheme which has better sensitivity to detect changes in the brain to help us fully understand just how the brain is affected by GWI.*

■ Our recent work on multi-component diffusion (Multi-D) assessments in a rat model of OP-induced GWI suggest an alternative in-vivo imaging approach for studying neuroinflammation [5].

- *Here, imaging parameters and mapping scheme of the Multi-D assessments were modified for the imaging of GW veterans.*

■ We applied multimodal imaging framework combining Multi-D and other common brain imaging measures to investigate followings:

- WM imaging features to characterize GWI
- GM microstructural and morphometric patterns specific to GWI
- Relationship between risk factors and brain integrity and connectivity alterations
- Relationship between imaging measures and self-report symptom scores

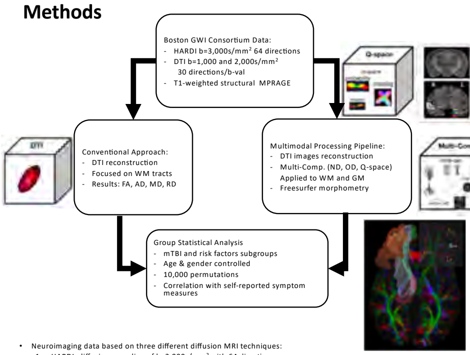
Participants

Table 1. Participants Demographics

	GWIC veterans (n=72)	Mean age
Healthy Controls (GW veterans)	15	53.58
Veterans with GWI	57	51.17
GWI Subgroups		
mTBI + Risk Factors	16	52.53
Without mTBI + Risk Factors	22	51.25

- GWI subjects defined based on Kansas case criteria.
- Kansas GWI criteria: The symptom domains are fatigue/sleep problems, somatic pain, neurological cognitive, mood symptoms, gastrointestinal symptoms, respiratory symptoms and skin abnormalities.
- Risk Factors: Pesticides and/or Fogged.

Methods



- Neuroimaging data based on three different diffusion MRI techniques:
  1. HARDI: diffusion encoding of b=3,000s/mm^2 with 64 directions.
  2. DKI: diffusion encoding of b=1,000s/mm^2 and 2,000s/mm^2 with 30 directions per each encoding.
- In addition to the diffusion MRIs, all subjects will have a high resolution T1-weighted structural images (MPRAGE) to provide macroscopic anatomical information.
- All scans have been obtained from an Achieva3T whole body MRI scanner (Philips Healthcare, Best, The Netherlands).

Results

Whole group (Con-GWI) analyses: GM & WM feature mapping

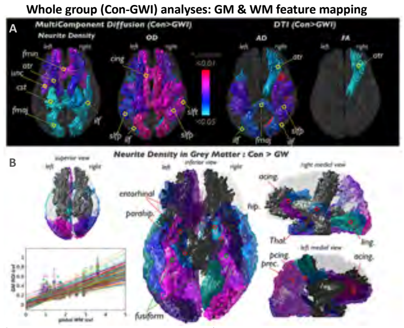


Figure 1. *Corrected P value mapped on a color spectrum, red representing p values less than 0.01 and light blue as 0.05.*
*(A) Reconstruction on white matter tracts with significant p values from both Neurite Density, orientation dispersion (OD) and DTI (right part). Figure shows group comparison results based on **mean tract values**.*
(B) Reconstruction on grey matter region of interests (ROIs) with significant P values from Multi-D processing (Neurite Density).
Graph: Trend of WM and GM correlation on neurite density.

◉ GWI showed decreased microscale (restricted/hindered) diffusivity in most of the major WM tracts.
- *Neurite Density: Highest group difference shown in anterior Callosal tract (fmin) & uncinate fasciculus.*
- *OD: : Highest group difference in anterior/posterior Callosal tract (fmin & fmax) and cingulum bundle.*

◉ Axial Diffusivity (lowered in GWI) was most sensitive index among all DTI measures.
◉ In GM, ND highlighted cortical/subcortical regions related to the limbic system.
◉ Subjects with lower WM microscale diffusivity have lower regional microscale diffusivity in the highlighted GM regions.

	White Matter Measures				Grey Matter Measures					
	Multi-D (ND/OD)		DTI (FA/MD/AD/RD)		Multi-D (ND/OD)		Multi-D (Q-Space) (r1/r3)		Volumetric (Vol/Thickness)	
Control - GWI	Fminor (ND & OD)	0.01^{OD} (2.5)	Fminor $(AD^{avg\_weight/avg\_center})$	0.02 (2.4)	Bilat_cau_aCing (ND & OD)	0.02^{OD} (2.4)	R_cau_aCing (r1 & r3)	0.04^{r1} (-2.0)	Bilat_cau_aCing (vol)	0.004 (3.0)
	Bilat_slft (OD)	0.02 (2.4)	Bilat_slft $(AD^{avg/avg\_weight}\ MD^{avg\_center})$	0.03 (2.3)	Bilat_rost_aCing (ND & OD)	0.003^{od} (3.0)	L_rost_aCing (r1 & r3)	0.01^{r3} (-2.6)	Bilat_supra_marginal (vol)	0.04 (2.1)
	R_ilf (ND & OD)	0.04^{OD} (2.1)	Bilat_ilf (AD^{avg/avg_weight})	0.001 (3.5)	Bilat_fusiform (ND)	0.02 (2.4)	Bilat_fusiform (r1 & r3)	0.02^{r3} (-2.5)	Bilat_postcent (vol)	0.0003 (3.6)
	Bilat_slfp (OD)	0.02 (2.4)	R_slfp $(AD^{avg/avg\_weight/avg\_center}\ MD^{avg\_center})$	0.02 (2.4)	R_hipppocampus (ND & OD)	0.03^{ND} (2.2)	R_hippocampus (r1 & r3)	0.008^{r3} (-2.7)	Bilat_middle_temp (vol)	0.01 (2.6)
	R_atr (ND & OD)	0.03^{OD} (2.3)	R_atr $(AD^{avg}\ MD^{avg\_center}\ FA^{avg})$	0.05 (2.0)	R_entorhinal (ND)	0.04 (2.1)	R_entorhinal (r1 & r3)	0.009^{r3} (-2.6)	R_trans_temp (vol)	0.01 (2.5)
Control - GWI with mTBI with risks	Bilat_unc (ND)	0.03 (2.3)	L_unc $(AD^{avg\_weight/avg\_center}\ RD^{avg/avg\_center}\ MD^{avg/avg\_weight})$	0.006 (2.7)	Bilat_rost_aCing (ND)	0.01 (2.7)	L_rost_aCing (r3)	0.04 (-2.2)	R_fusiform (vol)	0.05 (2.1)
	Bilat_ilf (ND)	0.03 (2.3)	R_ilf $(AD^{avg/avg\_weight}\ MD^{avg/avg\_weight})$	0.008 (2.9)	Bilat_post_cing (ND)	0.04 (2.3)	Bilat_post_cing (r1 & r3)	0.002^{r3} (-3.5)	R_parahipp (vol)	0.05 (2.1)
	Fminor (ND)	0.02 (2.5)	Fminor (AD^{avg_center})	0.04 (2.2)	R_paracentral (ND)	0.03 (2.3)	R_paracentral (r3)	0.04 (-2.2)	No sig.	
	Bilat_atr (ND)	0.03 (2.4)	Bilat_atr (MD^{avg_center})	0.02 (2.4)	R_banks_sts (ND)	0.03 (2.4)	R_banks_sts (r3)	0.02 (-2.5)	No sig.	
	Bilat_cst (ND)	0.04 (2.2)	R_cst (MD^{avg_center})	0.02 (2.5)	R_supra_marginal (ND)	0.04 (2.2)	R_supra_marginal (r1 & r3)	0.03^{r3} (-2.3)	No sig.	
Control - GWI without mTBI with risk	Bilat_atr (ND)	0.04 (2.1)	L_atr (AD^{avg_center})	0.05 (2.0)	R_lat_occipital (OD)	0.03 (2.3)	R_lat_occip (r1 & r3)	0.02^{r3} (-2.5)	Bilat_lat_occip (vol)	0.03 (2.3)
	Fminor (ND)	0.04 (2.2)	R_ilf $(AD^{avg/avg\_weight/avg\_center})$	0.003 (3.1)	L_rost_aCing (ND)	0.03 (2.3)	R_post_cing (r1 & r3)	0.02^{r3} (-2.5)	L_transverse_temp (th)	0.02 (2.4)
	Bilat_cst (ND)	0.04 (2.2)	No sig.		L_cuneus (ND)	0.03 (2.4)	R_inf_parietal (r1 & r3)	$0.03^{r1/r3}$ (-2.3)	No sig.	
	No sig.		No sig.		L_temp_pole (OD)	0.01 (2.7)	R_fusifom (r1 & r3)	0.04^{r3} (-2.2)	No sig.	
	No sig.		No sig.		L_isthmus_cing (OD)	0.03 (2.3)	R_banks_sts (r1 & r3)	0.008^{r3} (-2.9)	No sig.	

Table 2 *Whole group and subgroup statistical analysis across different measures. Each group statistics was performed with age and gender control and 10,000 permutations. Significant p and t (in parentheses) values are listed. White matter tracts or grey matter ROIs showing consistent significant group differences across measures are highlighted in yellow. Only top 5 or consistent significant tracts/ROIs are listed. In DTI section, we included statistics based on tract density weighted average tract value and mean tract center value in addition to mean tract value (shown in Fig 1A right). All GWI subjects defined by Kansas GWI case definition. CDC GWI definition group statistics also performed (data not shown).*

Abbreviations: ND = neurite density. OD = orientation dispersion, FA: fractional anisotropy, MD: medial diffusivity, AD: axial diffusivity, RD: radial diffusivity, Vol: volume. Bilat: bilateral. Cau: caudal, rost: rostral, lat: lateral, sup: superior, temp: temporal, aCing: anterior cingulate, cing: cingulate, inf: inferior, post: posterior, occip: occipital, postcent: postcentral, parahipp: parahippocampal, trans: transverse.

◉ More widespread group difference pattern was captured by Multi-D mapping in both WM and GM than the conventional measures (DTI, volumetry).
◉ Conventional measures overlap with Multi-D mapping (e.g., Bilat_cau_aCing volume).
◉ ND & r1/3 of GM revealed clear distinction between subgroups (also see *Fig 2*).

<supplementary>
- *Based on these measures, machine learning classifier showed the highest classification performance for identifying GWI cases in the multi-compartmental model (accuracy:89%) followed by DTI (62%).*
- *Kansas GWI criteria captured more regional GM and WM structures than CDC criteria (data not shown).*

Subgroup (Con-GWI_mTBI+risks) analyses: GM feature mapping

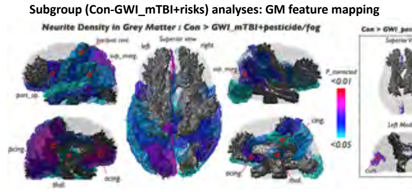


Figure 2. *3D brain mapping of significant statistical results from mTBI subgroup ND processing. Corrected P value displayed on a color spectrum, red representing p values less than 0.01 and light blue as 0.05. Exposure to either one or both risk factors (pesticide or fogged) were both taken into account. GWI_no_mTBI with risks subgroup (left panel) showed little group difference in the brain.*

◉ GWI subjects with more risk factors shows more consistent group differences (lowered in cases).
- *GM mapping highlights limbic and related areas (thalamus, cingulate cortex) only in mTBI+risks group.*
- *mTBI+risks group also showed more spread out microstructural alterations in peripheral GM regions.*
- *In WM, lowered ND in the fmin, bilateral thalamic tract and bilateral CST shown in both subgroups.*

Correlation (GWI – Kansas Subdomains) analyses: GM feature mapping

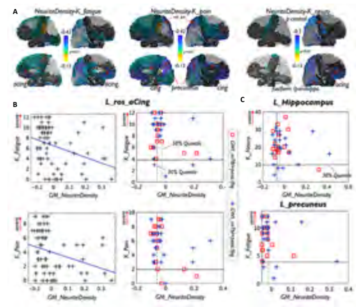


Figure 3. *3D brain visualization of significant statistical results from correlation between neurite density and Kansas subdomain measures. Pearson's linear correlation coefficient displayed as a color spectrum, from low (yellow) to high (blue).*
(A) Reconstruction on GM ROIs that show significant correlation between ND and subdomain scores. Three Kansas subdomains were assessed: fatigue, pain and neuro, from left to right, respectively.
(B) Representation of correlation curve fitting plot from left rostral anterior cingulate. Lower GM ND subjects revealed higher subdomain scores.
(C) Scatter plot representations from left hippocampus (limbic) and left precuneus. Lines show margin of 30% or 50% quantile in each axis, in lower 30~50% of ND, there is little subject in controls.

◉ GM ND measures explains Kansas subdomains better than other measures.
- *Midbrain limbic and related areas displayed the strongest negative correlation between GM ND and subdomain scores.*
- *Subjects with lowered ND had more severe illness symptom reports (pain, fatigue or neuro).*
- *More significant negative relationship between GM ND and subdomain symptoms shown in mTBI+risks group.*

Discussion

- More widespread and consistent alterations in the brain were captured by the multi-component diffusion measures and explained the level of self-report illness symptoms.
- Combined multimodal, microstructural diffusion and other imaging approaches reveal overall decreased GM values in GWI and specifically highlighting limbic and its related brain areas.
- Risk factor subgroup analyses revealed that multiple brain insults (mTBI + other risks) may cause more robust microstructural deteriorations in the brain.
- Multi-D mapping provided further description of GWI in terms of microstructural alterations which can be a potential imaging features for tracking inflammatory response in the brain[6].
- Further investigation is needed to see whether the robust alterations from multiple insults may result in further damage in later years of the veterans suffering GWI and thereby increase risks on either dementia[7] or other neurological illness.

This work is supported by a department of Defense CDMRP GWI research award (W81XWH-17-1-0440)

References:
1. O'Donovan et al. (2015). Altered inflammatory activity associated with reduced hippocampal volume and more severe posttraumatic stress symptoms in Gulf War veterans. Psychoneuroendocrinology, 51, 557–566.
2. Whistler et al. (2009). Impaired immune function in Gulf War Illness. BMC Medical Genomics, 2(1), 12.
3. Chao et al., (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 31:493–501.
4. Chao et al. (2015). Effects of low-level sarin and cyclosarin exposure on white matter integrity in Gulf War Veterans. NeuroToxicology, 48, 239–248.
5. Koo et al. (2018). Long-term effects of curcumin in the non-human primate brain. Brain Research Bulletin, 142, 88-95.
6. Koo et al. (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.
7. Barnes et al. (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. doi:10.1001/jamaneurol.2018.0815

Objective Biomarkers of Gulf War Illness: White Matter Microstructural Integrity, Cognition, and Blood Biomarkers in Gulf War Veterans

Clara G. Zundel[1,2], R. Killiany[2], B. Koo[2], M. Krengel[1,2], R. Toomey[3], J. Ajama[4], P. Janulewicz-Lloyd[4], M. Abreu[5], T. Heeren[4], E. Sisson[4], D. Little[6], L. Steele[6], N. Klimas[5] and K. Sullivan[4]

[1]VA Boston Healthcare System, [2]Boston University School of Medicine, [3]Boston University, [4]Boston University School of Public Health, [5]Nova Southeastern University [6]Baylor Medical College

Introduction

- Identifying objective biomarkers of Gulf War Illness (GWI) is one key focus area of the Boston GWI Consortium.
- Key symptoms of GWI include fatigue, chronic pain and cognitive problems.
- Our prior magnetic resonance imaging (MRI) and cognitive studies of GW veterans have found reduced brain white matter (WM) volumes and cognitive decrements in veterans with GWI compared to those without GWI (Sullivan 2003, Heaton 2007, Sullivan 2018).
- This study correlates cognitive, fatigue, sleep, and pain outcomes with brain volume, WM microstructural integrity and blood glutamate and phosphate levels in veterans with GWI.

Participants & Methods

- Participants included 72 GW veterans (57 GWI cases, 15 healthy controls).
- Cases and controls did not differ by age, sex or education (mean age 50yrs; mean education 15yrs).
- Assessments included a full cognitive battery, McGill pain questionnaire, Pittsburgh sleep quality Index, multidimensional fatigue inventory, as well as MRI brain imaging and blood glutamate and phosphate levels.
- Brain images were post-processed for structural volumes with Freesurfer software. For each WM pathway, we also obtained measures of average Fractional Anisotropy (FA), Axial Diffusivity (AD), Radial Diffusivity (RD), and Mean Diffusivity (MD).

Results

- ANCOVA volumetric comparisons showed significantly lower Total Cortex, Precentral Gyrus, and Superior Longitudinal Fasciculus (SLF) volumes in GWI cases vs. controls (all $p<0.05$) as shown in Table 1.
- DTI WM microstructural integrity measures (FA, AD, RD, and MD) were significantly different in cases and controls in the Anterior Thalamic Radiations, Corpus Callosum, and the Inferior and Superior Longitudinal Fasciculus as shown in Table 2.
- WM microstructural integrity measures were significantly correlated with cognitive performance (COWAT and DKEFS Color-Word Interference Test) (Table 3), fatigue, sleep, and pain (Table 4), blood glutamate and phosphate levels (Table 5).

Table 1. Brain Volume Differences

Brain Area	GWI Case Mean	Control Mean	P-value
Total Gray Matter	595744	645131	0.01
Total Cortex*	434566	452208	0.05
Precentral Gyrus*	24465	27626	0.02
Caudal Middle Frontal Gyrus	11595	13314	0.01
Pars-opercularis	7602	8207	0.05
Rostral Middle Frontal Gyrus	19208	20989	0.03
Superior Frontal Gyrus	48851	52995	0.03
Pars-triangularis White Matter*	6766	6084	0.01
Superior Long. Fasiculus White Matter Parietal Endings - RH*	1044	1271	0.005

*remains significant after controlling for Age, Gender, and Intracranial Volume

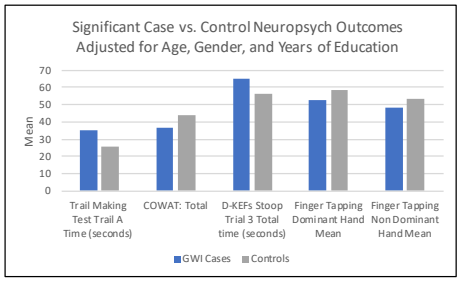


Table 4. WM Microstructural Integrity and Fatigue, Sleep, & Pain Correlates in GWI Cases

WM Pathway	Pain & Sleep Measurement	Volume	RD	MD	FA
Left SLF - Parietal	MFI-20 Fatigue Score		-0.306	-0.28	
Left SLF - Parietal	Pittsburg Sleep Quality Index Total		-0.308	-0.28	
Right SLF - Pareital	Pittsburg Sleep Quality Index Total		-0.306		0.298
Left SLF - Temporal Endings	McGill Pain Score				0.276
Left ATR	McGill Pain Score	-0.649			

Table 5. WM Microstructural Integrity and Blood Correlates in GWI Cases

WM Pathway	Blood Measure	Volume	RD	MD	FA
Right SLF - Parietal	Phosphate		0.296	0.272	-0.289
Left SLF - Temporal	Glutamate	-0.328			

Table 2. DTI Measure Significant Differences

WM Tract	DTI Measure	GWI (n=57)	Control (n=15)	p-value
Right ATR	FA	0.35937362	0.37088162	0.038
Right ATR	AD	0.00071428	0.00073031	0.0401
CC Forceps Major	MD	0.00051646	0.00053759	0.0132
CC Forceps Major	AD	0.0008684	0.00088951	0.0246
Left ILF	AD	0.00080914	0.00082825	0.0330
Right ILF	AD	0.00078995	0.00082085	0.0003
Right ILF	MD	0.00053538	0.00055274	0.0074
Right SLF - Parietal	AD	0.00069715	0.00071474	0.0174
Left SLF - Termporal	AD	0.00073479	0.00075100	0.0184
Right SLF - Temporal	AD	0.00070776	0.00072608	0.0138

ATR - Anterior Thalamic Radiation, CC - Corpus Callosum,
ILF - Inferior Longitudinal Fasciculus, SLF- Superior Longitudinal Fasciculus

Table 3. WM Microstructural Integrity and Cognitive Correlates in GWI Cases

WM Pathway	Cognitive Task	Volume	AD	FA
Left Cingulum - Angular Bundle	COWAT Total		0.281	
Right Cingulum - Angular Bundle	COWAT Total		0.299	
Left Cingulate Gyrus Endings	DKEFS Condition 3 Total Time (seconds)	-0.319		-0.27
Corticospinal Tract LH	DKEFS Condition 3 Total Time (seconds)	-0.301		
Left SLF - Temporal	DKEFS Condition 3 Total Time (seconds)	-0.309		

Discussion

- Results indicate that veterans with GWI perform more poorly on neuropsychological tests of psychomotor speed, inhibition, and verbal fluency compared to veterans without GWI.
- These neuropsychological differences are also correlated with WM microstructural integrity measures, as well as increased levels of glutamate and phosphate in the blood.
- These WM changes are an integral part of GWI pathobiology and the associated behavioral phenotype and should be investigated further in larger samples.

References

Heaton, K. J., Palumbo, C. L., Proctor, S. P., Killiany, R. J., Yurgelun-Todd, D. A., & White, R. F. (2007). Quantitative magnetic resonance brain imaging in US army veterans of the 1991 Gulf War potentially exposed to sarin and cyclosarin. *NeuroToxicology*, *28*(4), 761-769. doi:10.1016/j.neuro.2007.03.006

Sullivan, K., Krengel, M., Proctor, S., Devine, S., Heeren, T., & White, R. (2003). Cognitive Functioning in Treatment-Seeking Gulf War Veterans: Pyridostigmine Bromide Use and PTSD. *Journal of Psychopathology and Behavioral Assessment*, *25*(2), 95-103.

Sullivan, K., Krengel, M., Bradford, W., Stone, C., Thompson, T. A., Heeren, T., & White, R. F. (2018). Neuropsychological functioning in military pesticide applicators from the Gulf War: Effects on information processing speed, attention and visual memory. *Neurotoxicology and Teratology*, *65*, 1-13. doi:10.1016/j.ntt.2017.11.002

Funding: This study was funded by CDMRP award # GW120037

E-mail address: cgzundel@bu.edu

Mild TBI During the War is Associated with Further Microstructural Alterations in the Cortical Gray and White Matter in 1991 Gulf War Veterans with Gulf War Illness

Bang-Bon Koo, Ph.D.
Anatomy & Neurobiology

Disclosure

Presenter has no relevant financial or non-financial interest to disclose.

This continuing education activity is managed and accredited by AffinityCE in cooperation with the International Brain Injury Association. AffinityCE, IBIA, and all accrediting organizations do not support or endorse any product or service mentioned in this activity. Disclosure will be made when a product is discussed for an unapproved use.

AffinityCE staff, IBIA staff, planners, and reviewers have no relevant financial or non-financial interests to disclose.

Commercial support was not received for this activity.

Learning Outcomes

- Discuss potential impacts of mild Traumatic Brain Injury (mTBI) in the brain of Gulf War Veterans.
- Discuss neuroimaging methods available for studying mTBI in veterans.

How to Claim CE

Please visit the following URL to begin claiming credit:

https://ibia.cds.pesgce.com

Overview

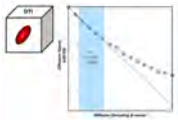
- Neuroimaging observations in 1991 Gulf War (GW) Veterans with a history of mild Traumatic Brain Injury (mTBI).
- Relationships to Illness Symptoms
- Relationships to Blood immune markers

Introduction

- Gulf War Illness (GWI), is characterized by a combination of symptoms including widespread pain, fatigue and neuropsychological impairments.
  - *These symptoms have been thought to be developed as a result of an innate immune response to a variety of different types of risk factors, such as toxic insults (e.g., organophosphates) or infection.*
  - *Persisting symptoms of GWI has been shown to coincide with a heightened or chronic inflammatory reaction*
- mTBI is common in veterans and has linked to increase risks of long-term neurodegenerations.
  - *Self-report of past mTBI during war was associated with GWI symptom severity.*
- In this study, we applied diffusion tensor imaging (DTI), Neurite density imaging (NDI) and Morphometry analysis on GW veterans to study whether mTBI during war resulted in more detrimental impacts to the brain.

Introduction

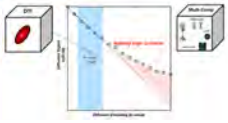
- Diffusion Tensor Imaging is typically performed using b-values ~1000s/mm^2 and the quantification of diffusivity is performed using linear modeling.



-DTI has been a standard measure for studying white matter anatomy and connections in-vivo.

Introduction

- Diffusion Tensor Imaging is typically performed using b-values ~1000s/mm^2 and the quantification of diffusivity is performed using linear modeling.

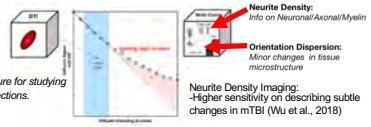


standard measure for studying
omy and connections.

- Neurite Density Imaging adds high b-value components and provides multi-compartmental modeling.

Introduction

- Diffusion Tensor Imaging is typically performed using b-values ~1000s/mm² and the quantification of diffusivity is performed using linear modeling.



-DTI has been a standard measure for studying white matter anatomy and connections.

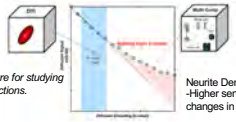
Neurite Density Imaging:
-Higher sensitivity on describing subtle changes in mTBI (Wu et al., 2018)

- Neurite Density Imaging adds high b-values and provides multi-compartmental modeling.

Mild TBI & GMWM Microstructural Alterations in 1991 Gulf War Veterans

Introduction

- Diffusion Tensor Imaging is typically performed using b-values ~1000s/mm² and the quantification of diffusivity is performed using linear modeling.



-DTI has been a standard measure for studying white matter anatomy and connections.

Neurite Density Imaging:
-Higher sensitivity on describing subtle changes in mTBI (Wu et al., 2018)

- Neurite Density Imaging adds high b-values and provides multi-compartmental modeling.
- In this study, we planned to combine these 2 different diffusion MRI techniques to study mTBI in GW veterans.

Mild TBI & GMWM Microstructural Alterations in 1991 Gulf War Veterans

Participants

Table 1. Participants Demographics

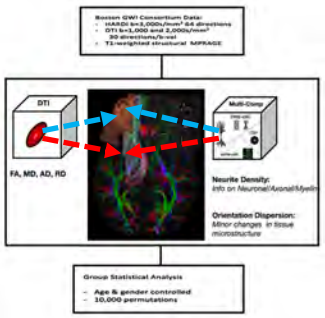
	GWIC veterans (n=68)	Mean age
Healthy Controls (GW veterans)	15	53.58
Veterans with GWI	53	51.17
	GWI Subgroups	
mTBI during war (labeled: mTBI)	20	52.53
Without mTBI (labeled: noTBI)	33	51.25

- GWI subjects defined based on ***Kansas case criteria***.
- Kansas GWI criteria: The symptom domains are fatigue/sleep problems, somatic pain, neurological cognitive, mood symptoms, gastrointestinal symptoms, respiratory symptoms and skin abnormalities.

Mild TBI & GMWM Microstructural Alterations in 1991 Gulf War Veterans

Methods

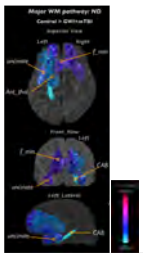
- All scans have been obtained from an Achieva3T whole body MRI scanner (Philips Healthcare, Best, The Netherlands).



Results:

Multi-component diffusion imaging in WM

Results: *Multi-component diffusion model (White Matter Connections)*

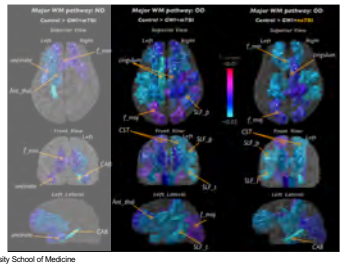


Lowered Neurite Density in GWI +mTBI group only.

Highest group difference pattern was shown in the anterior Callosal tract (f_min) & the uncinate fasciculus

-> connections between Ant. Frontal and temporal

Results: *Multi-component diffusion model (White Matter Connections)*

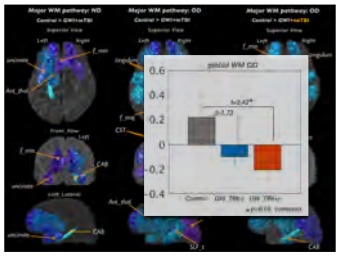


Lowered Orientation Dispersion in both GWI +mTBI and -noTBI groups.

GWI +mTBI had more widespread group difference pattern covering most of the major WM connections compared to the controls.

Highest in the posterior Callosal tract (f_maj) and SLF_temporal.

Results: *Multi-component diffusion model (White Matter Connections)*



Global WM OD showed clear distinction between groups.

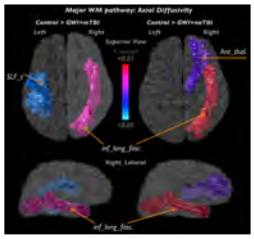
Results: *Diffusion Tensor Model*

Three major WM pathways highlighted from the Axial Diffusivity mapping.

Lowered Axial Diffusivity pattern was confirmed in the right inferior longitudinal fasciculus, left superior longitudinal (GWI +mTBI),

Lowered Axial Diffusivity pattern was also confirmed in The Right inferior longitudinal fasciculus and The right anterior thalamic tract (GWI -noTBI).

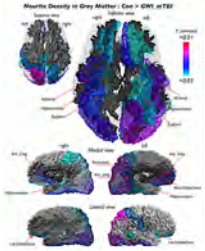
Interestingly, the difference pattern was more significant in GWI +noTBI group than the +mTBI.



Results:

Multi-component diffusion imaging in GM

Results: *Multi-component diffusion model in the Gray Matter*



Clear group differences were confirmed in the Grey Matter Neurite Density measures in GWI +mTBI group :

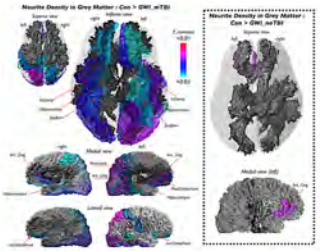
-lowered Neurite Density in the limbic system in GWI +mTBI group.

- *Cingulate*
- *Hippocampus*

And low Neurite Density regions connected to the limbic structures

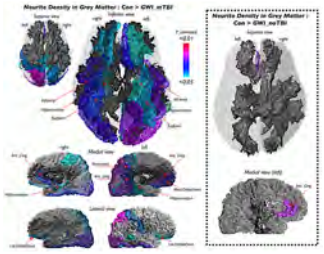
- *Med. orbito-frontal*
- *Fusiform / Inf-temporal cortex*
- *Medial Parietal cortex*
- *Lateral Occipital cortex*

Results: *Multi-component diffusion model in the Gray Matter*

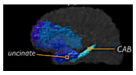


In GWI -noTBI group, lowered GM Neurite Density pattern was shown in the left ant. cingulate cortex compared to control veterans.

Results: *Multi-component diffusion model in the Gray Matter*

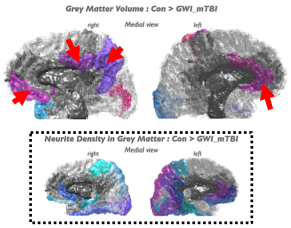


Taken together with the WM Neurite Density results,



GM ND patterns may indicate spread-out of microstructural damage in addition to the WM damage

Results: *Morphometry in the Gray Matter*



Additional regions were highlighted from local cortical volumetry:

Smaller volume in the structures related to the limbic system in +mTBI group compared to the controls:

- *Left ant. Cingulate cortex*
- *Right post. Cingulate cortex*
- *Right Med. Prefrontal / Parietal regions.*

Results:

Imaging, Illness Symptoms and Blood Immune Markers

Results: *Imaging and Symptoms*

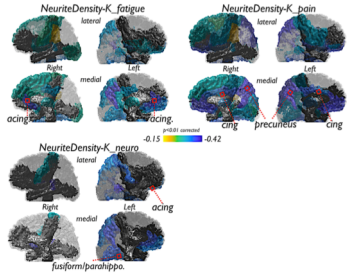
-GM Neurite Density measures had mild-to-moderate negative corelations to some of the Kansas sub-domain scores:

- *Higher score ~ more symptoms*

-> lowered Neurite Density related to severe symptoms

-> Highest correlations were confirmed in the Cingulate, medial prefrontal and inferior temporal regions had highest correlation.

WM measures did not correlated to the symptom scores.

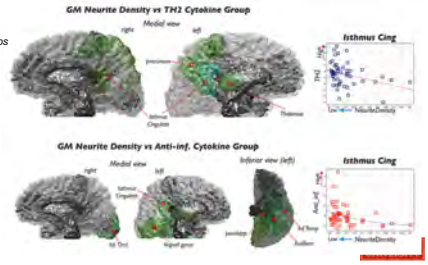


Results: *Imaging and Peripheral Immune measures*

GM Neurite Density mapping revealed negative relationships to some of blood cytokine groups:

-> lowered Neurite Density was related to the elevated TH2 and Anti- inflammatory cytokine markers

-> Regions covering the Thalamus, the Posterior Cingulate and the inferior temporal regions.

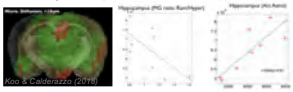


Discussions:

- Self report of past mTBI during war in the veterans with GWI was associated with ***more widespread and consistent White Matter alterations*** than the veterans without mTBI history.
- mTBI was associated with ***more consistent alterations in the limbic and its connected regions***.
- GM neurite density mapping ***explained the level of self-report illness symptoms*** and related to the ***elevation of the peripheral immune markers***.

Discussions

- In our previous imaging study on GWI rat models, enhanced micro-diffusivity mapping was correlated with active glial cell quantifications in the hippocampus.



- In GW veterans, lowered Neurite Density was also correlate with enhanced micro-diffusivity.
- Change in GM diffusion (i.e., lowered Neurite Density) may fingerprint the ***chronic impact of neuroinflammation*** and ***highlighting the regions vulnerable to further tissue damage*** in later life.

Discussions

- Further investigation is needed to see whether the robust microstructural alterations from mTBI may result in further damage in later years of the veterans suffering GWI and thereby increase risks on either dementia or other neurological illness.

Bio-imaging Informatics Lab.

(https://www.bumc.bu.edu/gnatneuro/research/bio-imaging-informatics-lab/)

Jasmine Cheng
Samantha Calderazzo
Wendy Guo
Alekha Kolli
Yi Guan
Man Yang
Woo-Sik Kim

Collaborators

Boston GWI Consortium

BU-SPH

Dr. Kimberley Sullivan

CDC

Dr. James O Callaghan

Baylor

Dr. Lea Steele

Nova

Dr. Nancy Klimas

BIDMC

Dr. Rebecca Edelstein

Boston VA

Dr. Margaret Hauser

FHS

Dr. Rhoda Au

SMC

Dr. Jae-Hun Kim

SMC

Dr. Young-min Shon

Mild TBI & GM/WM Microstructural Alterations in 1991 Gulf War Veterans

This work is supported by a department of Defense CDMRP GWI award (W81XWH-17-1-0440).

Thank you.

Boston University School of Medicine

In-vivo imaging of GWI

Bang-Bon Koo, PhD

Bio-imaging & Informatics Lab.
Boston University School of Medicine
August 18, 2020

Imaging GWI

In-vivo imaging of immune response in the brain can be a key to study Neuroimmune model of GWI

Different types of risk factors
Neurotoxicants,
Stressors,
Injury…

→ *heightened or chronic inflammatory reaction: Neuroinflammation* →

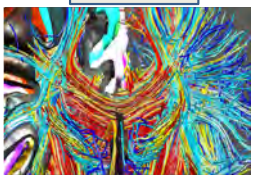
Variety of symptoms
Fatigue, Pain
Memory,
Gastrointestinal,
and so on…

"Our goal was to develop an objective imaging biomarker"

The 2nd Generation Diffusion MRI

The key concept on the GWIC imaging design was to utilize the 2nd generation diffusion MRI.

DTI



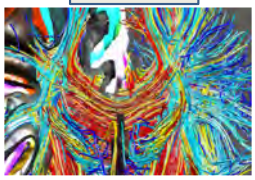
White matter modeling
Cortico-cortical connections
WM degeneration

2nd Gen Multi-B dMRI

The 2nd Generation Diffusion MRI

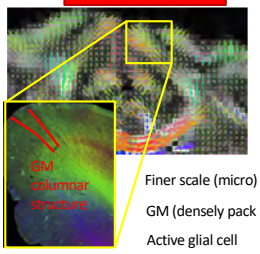
The key concept on the GWIC imaging design was to utilize the 2nd generation diffusion MRI.

DTI



White matter modeling
Cortico-cortical connections
WM degeneration

2nd Gen Multi-B dMRI



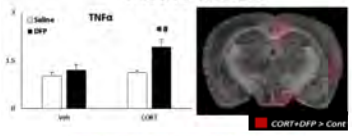
Finer scale (micro)
GM (densely pack region)
Active glial cell
Subneuronal components
e.g.) synaptic loss

The 2nd Generation Diffusion MRI

2nd Gen diffusion MRI in Animal model

DFP+CORT exposure to Rat Brain (O'Callaghan, 2015)

Acute model



Chronic model

Persistent patterns

Brain, Behavior, and Immunity 67 (2018) 42–46

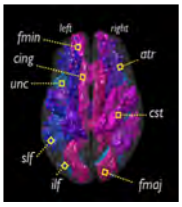
Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness

Bang-Bon Koo, Lindsay T. Michalovicz, Samantha Calderazzo, Kimberly A. Kelly, Kimberly Sullivan, Ronald J. Killiany, James P. O'Callaghan

Diffusion MRI in GW veterans

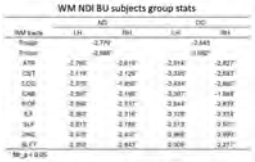
White matter assessments

All Subjects	GW Control	GWI Case
N	22	124
Age (years)	54.84	52.58
Gender (F/M)	3/19	25/99
Exposure to risk factors during war (% exposed)		
Chemical/Biological Warfare Agents	3	52



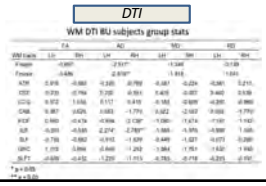
Multi-B dMRI

WM NDI BU subjects group stats



DTI

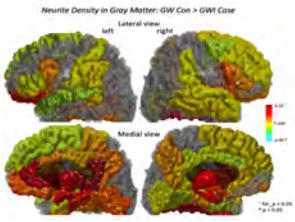
WM DTI BU subjects group stats



Diffusion MRI in GW veterans

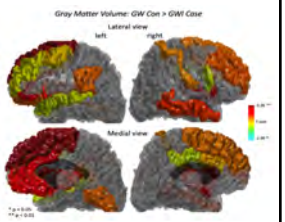
Grey matter assessment

Multi-B dMRI



Limbic/Paralimbic regions

Morphometry



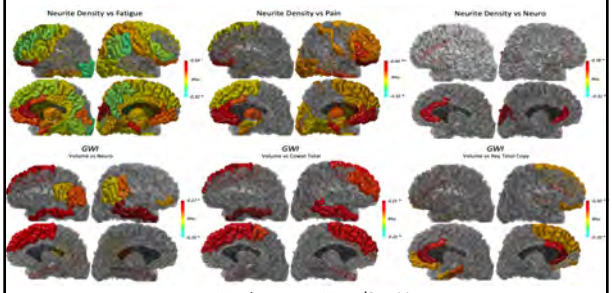
Not sig. under FDR

:Which communicates with autonomic nervous system, endocrine system and the viscera.

Diffusion MRI in GW veterans

Diffusion vs Kansas Domains

GM diffusion measures ~ Kansas Fatigue, Pain, Gastro domains

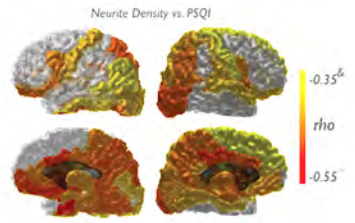


Morphometry vs Neuro/Cognitive

: indicating different neurological basis for different symptoms?

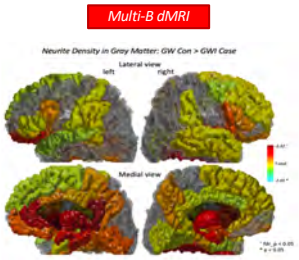
Diffusion MRI in GW veterans

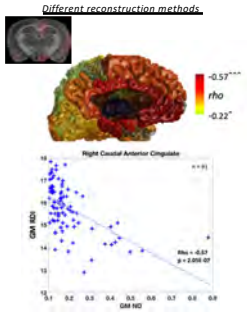
Diffusion vs Sleep deprivation



Diffusion MRI in GW veterans

ND vs Other multi-B diffusion reconstruction methods

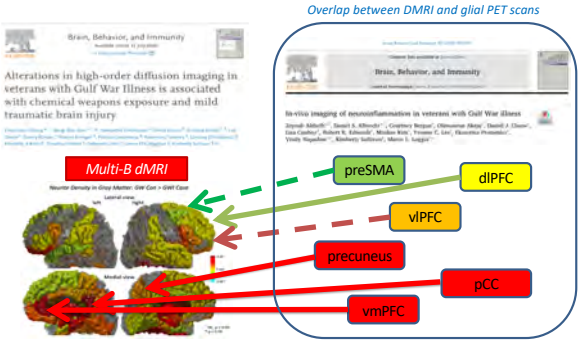




Also, consistent with DKI, FW

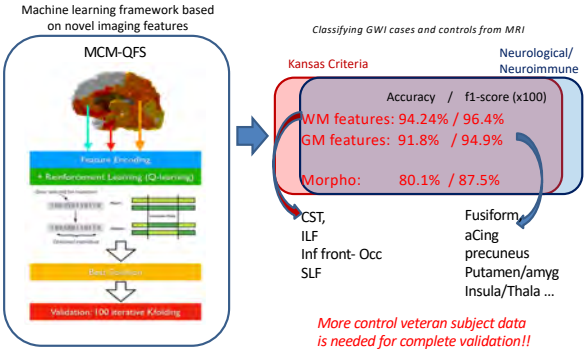
Diffusion MRI in GW veterans

The 2nd Gen DMRI and TSPO PET



MRI markers for single subject level diagnosis

"Can MRI markers from GWIC predict Kansas criteria?"



Conclusion

Change in microstructural diffusivity measure may fingerprint the ***chronic impact of neuroinflammation*** and ***highlighting the regions vulnerable to further tissue damage*** in later life.

- Lowered microstructural diffusivity : widespread WM and Limbic/paralimbic GM
- Better distinction between GWI cases and controls than morphological markers.
- Explaining Kansas Fatigue, pain and Gastro domain scores.
  However, Neuro/cognitive measures were better explained in Morphometrical measures
- Difference pattern overlaps to the GWI animal model study and TSPO pet data.
- Diffusion markers provided promising results in a single subject level classification trial.
  More control subject data is required for further investigation.

Acknowledgements

This work is supported by a department of Defense CDMRP GWI award (W81XWH-17-1-0440) and GWI consortium award (W81XWH-13-2-0072).

Thank you!

Bio-imaging Informatics Lab.
(https://www.bumc.bu.edu/anatneuro/research/bio-imaging-informatics-lab/)

PI: Bang-Bon Koo (bbkoo@bu.edu)

Jasmine Cheng
Yi Guan
Weifan Chen
Samantha Calderazzo
Wendy Guo
Sophia Koo
Jason Hong
Christian Arthur
Prinjali Kalyan
Chancellor Lee

Collaborators

BU-SPH

Dr. Kimberley Sullivan

Dr. Timothy Heeren

CDC

Dr. James O'Callaghan

Baylor

Dr. Lea Steele

MGH

Dr. Marco Loggia

Nova

Dr. Nancy Klimas

BIDMC

Dr. Rafeeque Bhadelia

SMC

Dr. Jaehoon Kim

HYU lecture

Bio-imaging Informatics Lab.

Koo, Bang-Bon PhD

1

Research Network in BU

Bio-imaging Informatics Lab.

Aging / Alzheimer's Disease & Dementia

In-vivo Imaging Methods & defining imaging biomarkers

Virtual Dissection of the brain in different scales.

Tools for measuring the Brain: tracking changes in Structure and Function Mapping and Modeling

Informatics

Computational methods on effective use of biomedical imaging data for addressing scientific problems and decision making on human health issues.

2

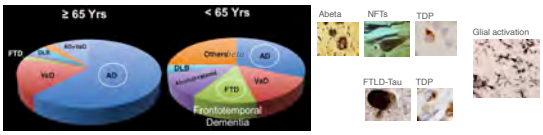
Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: "an inflammatory response in CNS"

"뇌의 노화 과정"을 이해하는 데의 중요한 요소



3

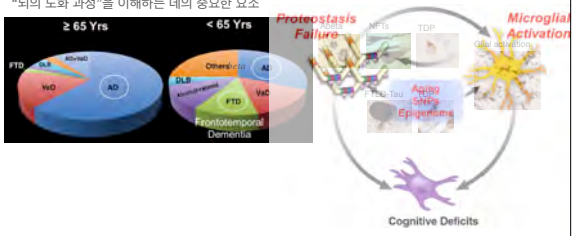
Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: "an inflammatory response in CNS"

"뇌의 노화 과정"을 이해하는 데의 중요한 요소



4

Research Network in BU

Bio-imaging Informatics Lab.

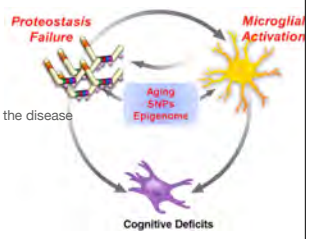
In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: "an inflammatory response in CNS"

Neuron Centric Hypothesis
Proteostasis malfunction -> neuronal injury

vs.

Immune Cell centric Hypothesis
Maladaptive innate immune cells -> the driver of the disease
Next generation therapeutic strategy

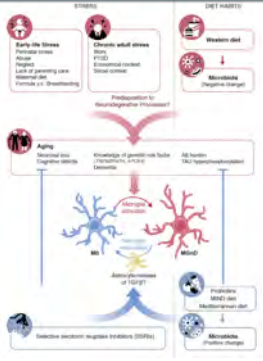


5

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation



Madore et al., 2019

6

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

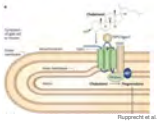
Neuroinflammation: In-vivo Imaging

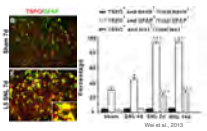
TSPO PET imaging

The Translator protein (18 kDa)

A five transmembrane domain protein in the outer mitochondrial membrane

Upregulated by activated glial cells (HIV, AD, pain)





7

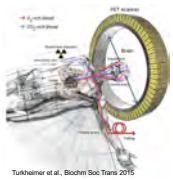
Research Network in BU

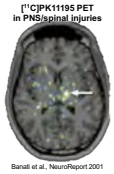
Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: In-vivo Imaging

TSPO PET imaging





1st gen.

[11C]PBR28
80x higher specific binding
(Kreisl et al., 2020)

8

Research Network in BU

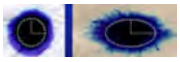
Bio-imaging Informatics Lab.

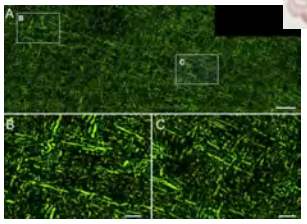
In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: In-vivo Imaging

Diffusion MRI

Cellular water diffusivity
(Motion of water through tissue)





9

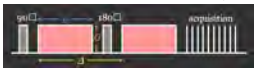
Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

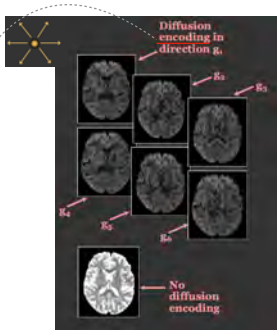
Neuroinflammation: In-vivo Imaging

Diffusion MRI



Diffusion speed 혹은 scale,

# of encoding direction,



10

Research Network in BU

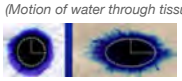
Bio-imaging Informatics Lab.

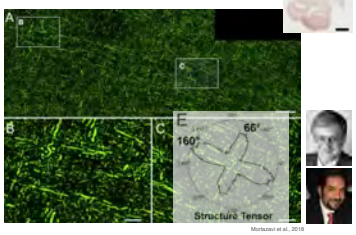
In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: In-vivo Imaging

Diffusion MRI

Cellular water diffusivity
(Motion of water through tissue)





11

Research Network in BU

Bio-imaging Informatics Lab.

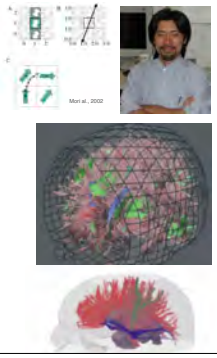
In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

12

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

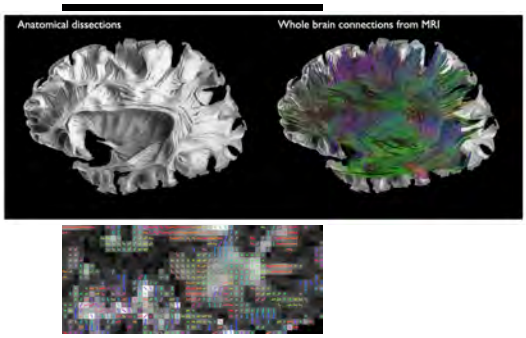


13

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**



14

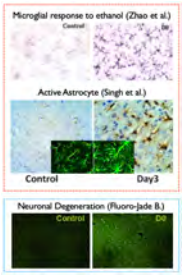
Research Network in BU

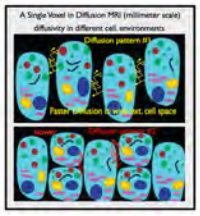
Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

Neuroinflammation: In-vivo Imaging

Complex Diffusion MRI





15

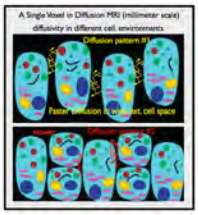
Research Network in BU

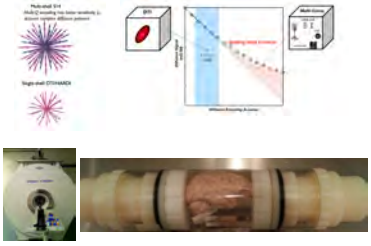
Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

Neuroinflammation: In-vivo Imaging

Complex Diffusion MRI



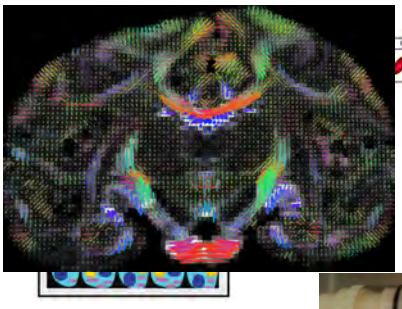


16

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

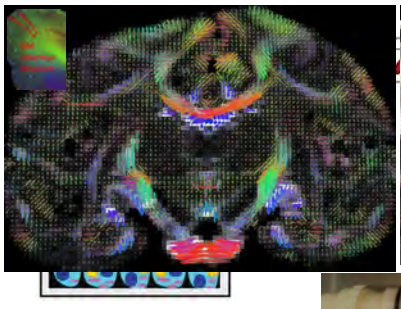


17

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

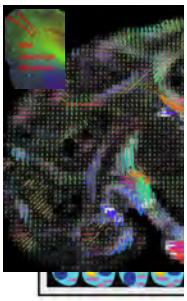


18

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**



Adding Ex-vivo Connectional Inference

comparing ex-vivo tract tracing results with in-vivo connections would be helpful to determine optimal reconstruction parameters

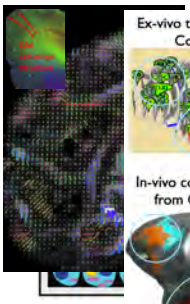
CoCoMac is a Web-based database of the connectivity between brain regions in the rhesus macaque.

19

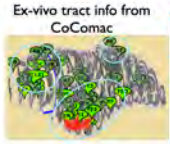
Research Network in BU

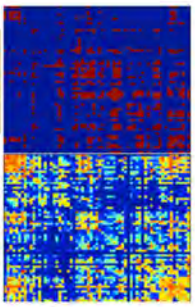
Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**



Ex-vivo tract info from CoComac





In-vivo connectivity info from CBI toolbox

ıl Inference

cing results with in-vivo
ul to determine optimal

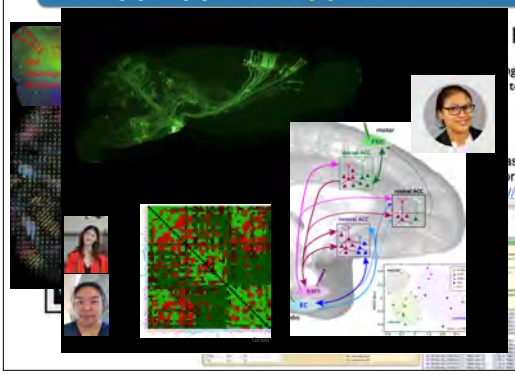
based database of the
ı brain regions in the

20

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**



Inference

g results with in-vivo
to determine optimal

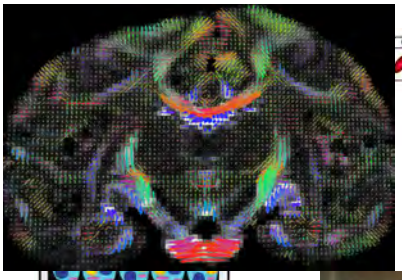
ased database of the
rain regions in the
//cocomac.g-node.org

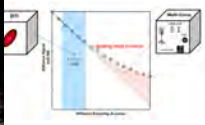
21

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**





22

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

Neuroinflammation: In-vivo Imaging (Gulf War Illness Model)

Dr. Kimberly Sullivan (BU SPH)

•Medically unexplained chronic symptoms (fatigue, pain/headaches, memory issues, gastrointestinal and sleep disorders, etc).

•Exposure to **neurotoxicants** (Pyridostigmine bromide, DEET, sarin) may have role (White et al., *Cortex* 2016).

•Neurotoxicants induce a neuroinflammatory response, that can be enhanced by physiological stressors

•Neuroinflammation can produce **sickness behaviors**

23

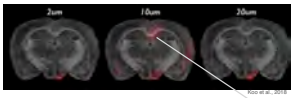
Research Network in BU

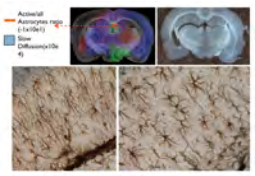
Bio-imaging Informatics Lab.

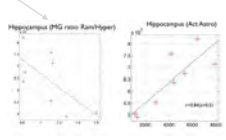
In-vivo Imaging & imaging biomarkers: **Imaging Neuroinflammation**

Dr. James O'Callaghan (CDC)

Neuroinflammation: In-vivo Imaging (Gulf War Illness Model)







Samantha Calderazzo

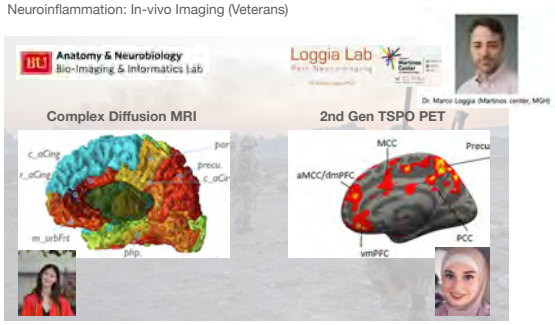
24

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation

Neuroinflammation: In-vivo Imaging (Veterans)

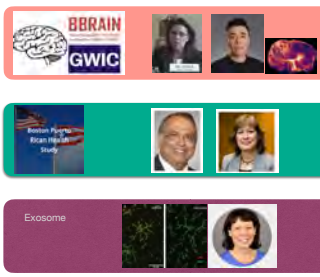


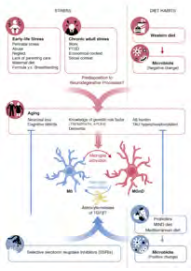
25

Research Network in BU

Bio-imaging Informatics Lab.

In-vivo Imaging & imaging biomarkers: Imaging Neuroinflammation





Madore et al., 2019

26

Research Network in BU

Bio-imaging Informatics Lab.

Aging / Alzheimer's Disease & Dementia

In-vivo Imaging Methods & defining imaging biomarkers

Virtual Dissection of the brain in different scales.

Tools for measuring the Brain: tracking changes in Structure and Function Mapping and Modeling

Informatics

Computational methods on effective use of biomedical imaging data for addressing scientific problems and decision making on human health issues.

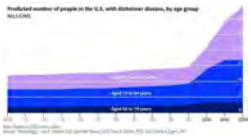
27

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

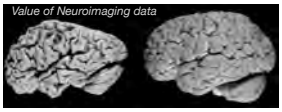


Personalized Medicine:

Prognosis

&

Cure



28

Research Network in BU

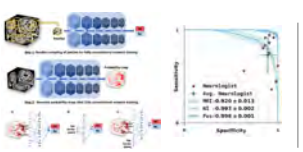
Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

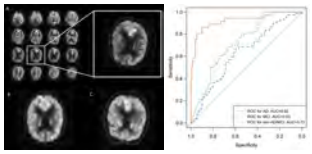
Phenotyping neurodegeneration using Machine Learning

Classifying Alzheimer's Disease using ML: 어디까지 왔나?

MRI



PET



29

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Classifying Alzheimer's Disease using ML: 어디까지 왔나?

Remaining Issues

Not so big data problem

Simple handwriting digit recognition
MNIST database
60,000 samples for 10 classes

Imaging data: 3 class problem / less than 2,000

Multi-class???

AD vs. FTD vs. other types

PET: practicality

치매 예측?

30

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease

Mild Cognitive Impairment (MCI)

- The prodromal stage of dementia (in particular Alzheimer's disease)
- Highly heterogeneous disease trajectory, a large portion of MCI does not progress even after 10 years (Mitchell & Shiri-Feshki, 2009)

Need to identify MCI subjects with higher risk of progression: prognostic markers of AD

31

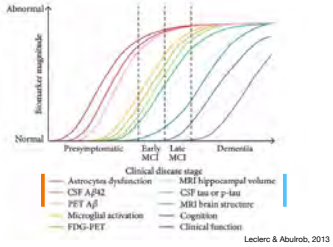
Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease



Leclerc & Abulrob, 2013

32

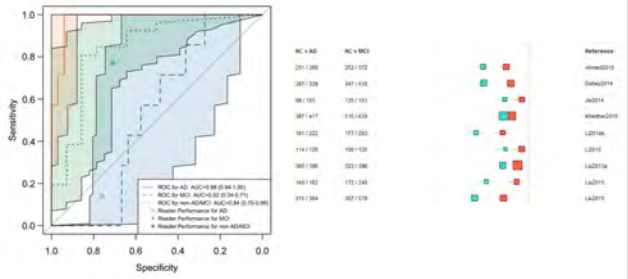
Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: MCI



33

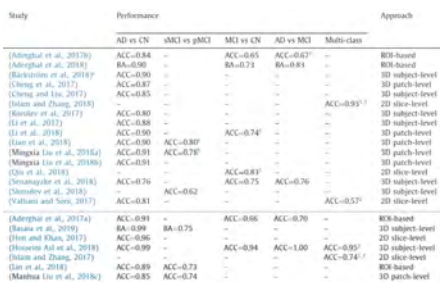
Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts



34

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

Deep Learning

Not working well on AD prognosis

Small data: yes

Inappropriate CNN framework: maybe

Missing link between neuroimaging and ML methods: only a few conventional imaging features are being tested

35

Research Network in BU

Bio-imaging Informatics Lab.

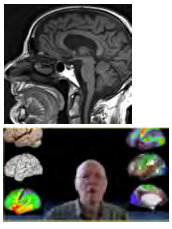
Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

#1 different ways to do quantitative measurements on this old image

#2 Deep learning for MRI



36

Research Network in BU

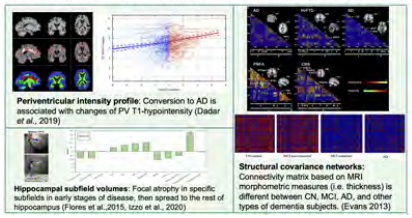
Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

Candidate features that may improve classification performance for AD prognosis



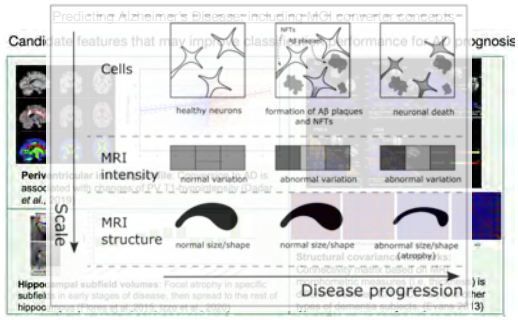
37

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning



38

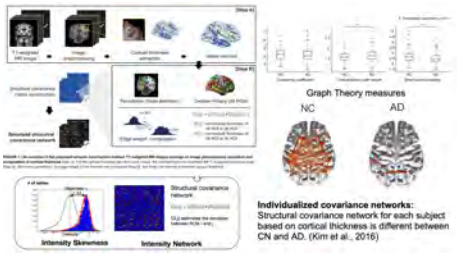
Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts



39

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

40

Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

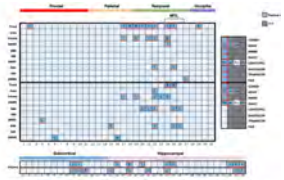


Table 5. Summary of MCI converters vs MCInc classification performances. This table compares the performances of classifiers using different input features. ACC: accuracy, SEN: sensitivity, SPE: specificity.

Input Measures	MCIc vs MCInc.			MCI vs. CN		
	ACC	SEN	SPE	ACC	SEN	SPE
sMRI + Cognitive	**91.67%**	93.67%	86.47%	97.35%	97.08%	99.1%
sMRI	90.11%	93.63%	79.53%	83.10%	83.9%	82.04%
Cognitive	82.67%	88.68%	61.63%	**98.81%**	99.28%	98.16%
Conventional sMRI only	88.68%	90.49%	75.25%	77.22%	74.21%	81.23%

41

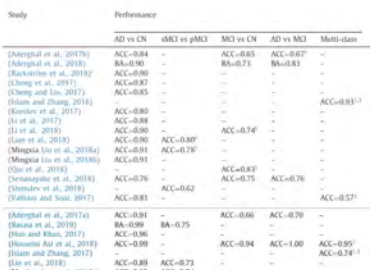
Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Deep Learning

Predicting Alzheimer's Disease: including MCI converter concepts



42

Research Network in BU

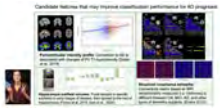
Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

1. Automation:

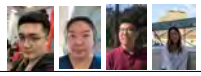


NEUROVAULT

BALSA

2. AD vs FTD

3. Merging Databases



43

Research Network in BU

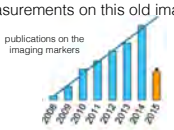
Bio-imaging Informatics Lab.

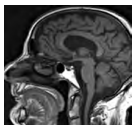
Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

#1 different ways to do quantitative measurements on this old image





#2 **Deep learning for MRI**

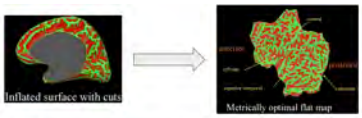
44

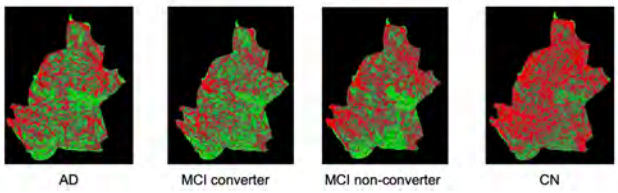
Research Network in BU

Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning





45

Research Network in BU

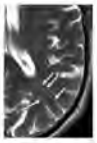
Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

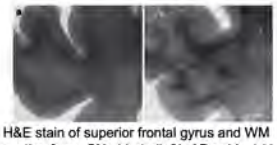
Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

Perivascular space (PVS): responsible for the clearance of interstitial fluid and waste from the brain, particularly during sleep (Wardlow et al., 2020)

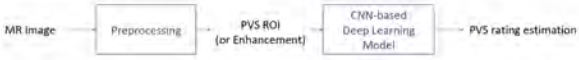


PVS on T2w MRI



H&E stain of superior frontal gyrus and WM section from: CN elderly (left), AD subject (right)

• Overall procedure



46

Research Network in BU

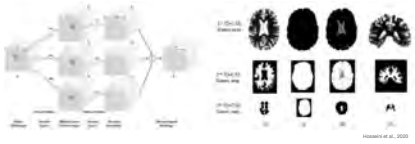
Bio-imaging Informatics Lab.

Informatics: Neuroimaging markers for Dementia

Phenotyping neurodegeneration using Machine Learning

Predicting Alzheimer's Disease: including MCI converter concepts

- For ADNI: 0.76 to 0.89 for the AD vs CN, 0.69 to 0.74 for sMCI vs pMCI. Similar to studies without data leakage: 0.76 to 0.91 for AD vs CN and 0.62 to 0.83 for sMCI vs pMCI.
- three approaches (3D subject-level, 3D ROI- based, 3D patch-level) provided similar performances, 2D slice was less sufficient
- With the sample size in ADNI, CNNs did not provide better performance compared to SVM



47