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14. ABSTRACT
We employed a novel classification framework based on a combination of brain connectomics and immuno-genetic approaches of Gulf War Illness (GWI) to develop novel computer-based diagnostic systems and features. In this project year, we confirmed that veterans with GWI have clear group specific microstructural alteration in limbic/paralimbic regions compared to GW veterans without illness. Multimodal imaging measures in the highlighted cortical regions had important role in improving classification performances. Our results also showed that self-report mile Traumatic Brain Injury, depression or Post-Traumatic Stress Disorder symptom profiles have strong diagnostic values for classifying neurological deteriorations in GW veterans when they combined to the Kansas criteria. In the following project year, we will continue to update subject data and work on building the classifiers to enhance the classification outcomes.

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

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

Almost 200,000 veterans returning from the 1991 Gulf War (GW) presented with multiple illness symptoms, such as fatigue, cognitive dysfunction, chronic pain, gastrointestinal issues, and other symptoms. These symptoms are thought to have developed as a result of an innate immune response to variety of different types of risk factors, such as toxicant insults or injury. Moreover, persisting symptoms of GW illness (GWI) has been shown to coincide with a heightened inflammatory reaction in the brain. Unlike a typical inflammatory response that resolves over time with slow but steady recovery of the immune system, the inflammatory response in GW veterans with illness symptoms appears to be chronic. This converging information suggests that a better understanding of brain-immune interactions may provide a key to unlocking the biological origin of GWI. In this project, we are employing a novel classification framework based on a combination of brain connectomics and immuno-genetic approaches of GWI to develop novel computer-based diagnostic systems and features. Biomarkers from different body systems affected in GWI including brain and immune functions may reflect different yet connected aspects of the disease. Incorporating joint distributional information across different biomarkers for decoding brain-immune interactions can provide a better understanding of GWI etiology over methods using a single marker or a simple concatenation of markers. We planned to utilize a machine learning framework to incorporate different biomarkers (blood tests, cerebrospinal fluid, cognitive tests and brain imaging) for further investigation of the complex interactions that represent GWI etiology. This project will also contribute to the establishment of the next generation of databases which can offer quantitative diagnostic information to the individuals who are suffering from GWI and plan for effective interventions at a more personalized level.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Gulf War Illness, White matter integrity, Gray matter microstructure, Brain mapping, Morphometry, Neuroinflammation, MRI biomarkers, Cognitive tests, Machine learning, Blood Cytokine, Mild Traumatic Brain Injury, Depression, Post Traumatic Stress Disorder, Chemical exposure, Pesticides, Kansas criteria, CDC criteria, Sleep quality measure, Pain, Fatigue

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major goals and tasks in the project year 2:

Major Goals & Research Specific Tasks	Timeline	Status	Re-action plan
Major Task 2: Build a unimodal classifier of GWI neuroimaging data	Months		
Subtask 1: Diffusion map reconstruction Boston data: DTI, GQI on DKI, GQI on HARDI, DKI, MicroD (data from 175 GW veterans) Texas data: DTI (data from 75 GW veterans)	4-10	• Aug 27, 2019: Achieved on all of the currently transferred data from GWIC (total 113 subjects).	• GWIC is using new recruitment efforts to complete recruitment goals including media and social media presence. • 55 Subjects Imaging data is recently collected from Huston site and will be transformed to the research team during 25th Project month.
Subtask 2: Cortical modeling and network reconstruction - includes visual inspection and corrections (data from 250 study participants; 150 cases, 100 controls)	4-10		
Subtask 3: Build classifiers per each diffusion map	11-18	• Aug 14, 2019: Classifiers (DTI, Q-space, multi-compartmental diffusion) were built from 91 subject data (BU site data)	• Classifiers will be updated after additional 55 subject data processing work done.
Milestone(s) Achieved: 1. high quality post-processed data 2. neuroimaging classifiers and their benchmark tests 3. feature descriptions – including 3D brainmap of GWI	4-18	1. Achieved on the BU data. 2. Achieved on the BU data. 3. Initial marker selection done on the existing BU site data.	• All newly acquired data will be promptly processed and updated.
Major Task 3: Build a unimodal classifier of GWI neuroimmune marker features			
Subtask 1: Build a classifier of CSF immune markers (50 GW veterans; 25 cases, 25 controls)	11-18	• Pending, only 7 CSF samples obtained to date from GWIC call-back study	• Alternative markers on CNS immune function (PET/resting fmri) will be combined and tested in the study.
Subtask 2: Build a classifier of blood immune markers (250 GW veterans; 150 GWI cases, 100 controls)	11-18	• Aug 14, 2019: Classifier was built from 91 subject data	• Classifiers will be updated after additional 55 subject data processing work done.
Milestone(s) Achieved: 1. classifiers and their benchmark tests (sensitivity of GWI). 2. descriptions of important features.	11-18	• Achieved on the existing data. • Results presented in the scientific meeting.	• All newly acquired data will be promptly processed and updated.
Major Task 4: Build multi-modal classifiers & decode brain-immune interactions (cont'd to project year 3)			
Subtask 1: Build a multi-modal classifier combining different neuroimaging markers of GWI	19-21	• Aug 27, 2019: Multimodal classifier (Morphometry, DTI, Q-space, multi-compartmental diffusion) was built from 91 subject data	• All newly acquired data will be promptly processed and updated.

Continued,

Major Goals & Research Specific Tasks	Timeline	Status	Re-action plan
Subtask 2: Build a multi-modal classifier of neuroimaging & blood immune markers (data from 250 GW veterans; 150 cases, 100 controls)	22-24	• Aug 27, 2019: Multimodal classifier (Morphometry, DTI, Q-space, multi-compartmental diffusion) was built from 91 subject data	• All newly acquired data will be promptly processed and updated.
Milestone(s) Achieved: 1. multi-modal GWI classifiers and their benchmark tests 2. descriptions of important features and their relationships		• Achieved on the existing BU site data • Results presented in the scientific meeting and GWIC study meeting	• All newly acquired data will be promptly processed and updated.

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:

Major activities in this project year include the followings: 1) Updating processing pipeline and defining neuroimaging biomarkers for GWI, 2) build unimodal classifiers based on neuroimaging and 3) immune biomarkers, 4) benchmark tests across different classifiers, 5) analyses on key factors for better classifier performances, 6) build multimodal classifier, 7) preparation work for building graphic user interface software.

The following components were added to the updated image processing pipeline:

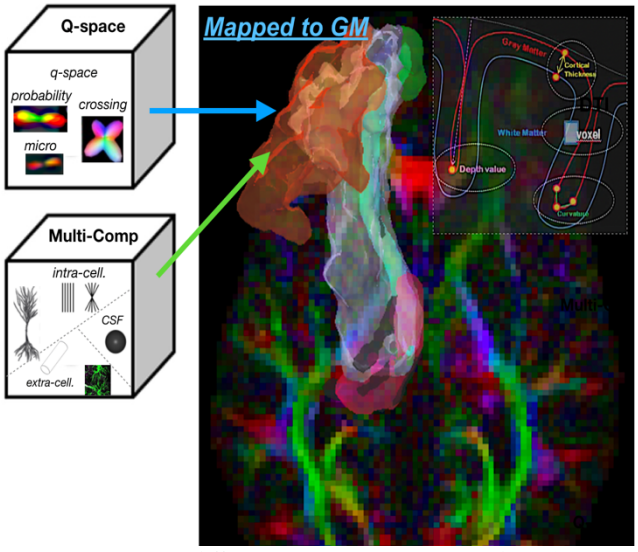

Figure 1. Gray matter diffusivity mapping. Q-space indices (microD, GFA) and multicomponent diffusion (ND, OD, ISO) indices are mapped to each of cortical GM structure (orange volume). These indices can be directly compared to morphometric measures (diagram, right side)

-Multi-dimensional diffusion mapping in the gray matter (GM): Cortical GM diffusivity mapping, which principle investigator (PI, Dr. Koo) developed in 2009 (Koo et al., 2009, 2010), has brought a novel perspective on diffusion MRI as a sensitive measure on in the grey matter microstructure and has served as the foundation of numerous studies. The research team applied this mapping strategy to the processing pipeline to investigate

microstructure in the GM. GM diffusion modeling parameters were empirically determined by iterative parameter selection method based on the maximum likelihood estimation of modeling fitting error. From q-space imaging, micro-diffusivity (microD) was calculated in 78 regions of interests (ROIs) in the GM and subcortical GM. From multi-component diffusion imaging, intracellular diffusion fraction (ND) and orientation dispersion (OD) was extracted in each of cortical and subcortical GM ROIs. Formal index provides a fraction of tissue composed of axons or dendrites and later index provides spatial configuration of the neurite structures in the GM. By adding these measures, macroscopic morphological information could be directly compared to the microscopic diffusion measures in the GM.

-Two machine learning classification methods were added to the pipeline: we added a multi-step random forest (RDF) classifier. In this approach, initial RDF classification was applied to train the classifier on the whole brain imaging measures in order to identify the first level feature weights for each connection. The first level feature weights represent the amount of contribution of each features to discriminate group differences. Features selected from initial RDF were used for training of the second level RDF classifier to iteratively find a subset of features among the first level features for best performance of the second level RDF.

Second approach we added to the pipeline was RDF based on canonical correlation framework RDF-CCA). RDF-CCA use hyperplane splits in the projected data space defined by canonical correlation analysis. This approach has some advantages over methods using axis-aligned decision boundaries (e.g., RDF) on followings: less infected to the classification parameter tuning, improved predictive accuracy and faster training times. The research team consulted the inventor of this approach (Dr. Rainforth at Oxford University) about potential technical considerations for applying this approach to the neuroimaging data. Also, feature importance measurement was added to the RDF-CCA framework after the discussions.

-Hippocampal subfield parcellation was added to the pipeline. We are processing hippocampal subfield volumetry on gulf war veteran's data now.

2. Specific objectives:

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 applied multivariate classification scheme to incorporate relationships between different biomarkers from different biological domains (imaging to cytokine markers) to investigate meaningful insight into GWI etiology. In addition, we also tested different classification designs based on adding additional symptom information (e.g, depression, PTSD) to determine interlink markers and symptoms relevant to the GWI. Overall, defining key features and cross-validating different types of machine learning classification methods were the specific goals for this project year.

3. Significant results:

In the project year 2, neuroimaging data collection has still yet to reached the planned sample size (175 from Boston site, 75 from Texas site). By end of Aug, 91 subject data from the Boston site was processed and used for statistical or machine learning analysis. From the other site in Houston, 22 subject data was transferred and processed for neuroimaging measures. However, we did not add

this data to the statistical or machine learning analysis. We will combine this data after having additional 55 subject data transferred from the other site. Significant results in this report were based on the existing BU site data (16 controls vs 75 GWI). In the following sub-sections, we listed several important information achieved in the project year 2.

3A. GWI veterans have widespread microstructural alterations in WM.

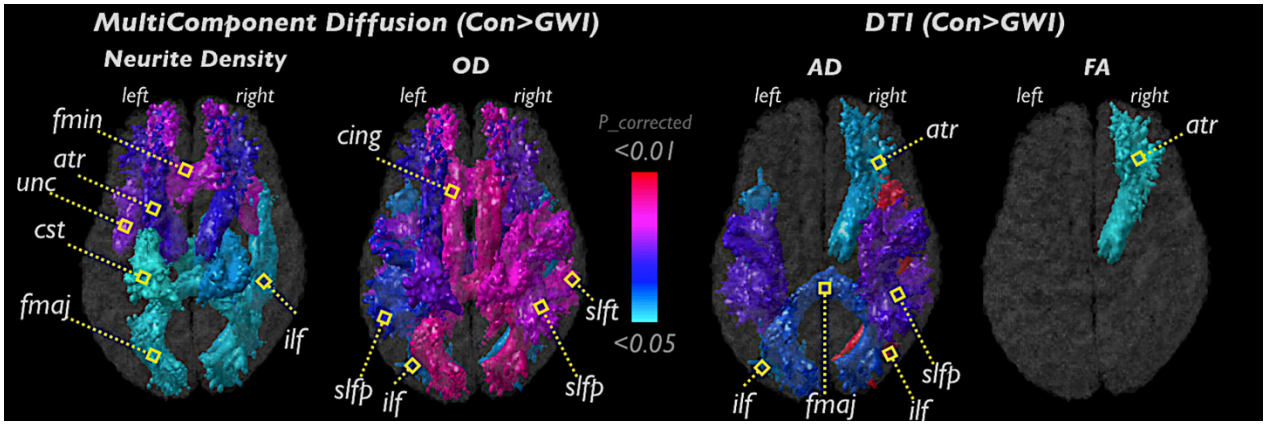

Figure 2. Multicomponent diffusion and diffusion tensor imaging in WM. Significant group differences between controls (Con) and GWI veterans are depicted in color coded (colorbar in middle) WM volume renderings. Fmin: anterior corpus callosal bundle, atr: anterior thalamic neucleus, cing: cingulum bundle, unc: uncinate fasciculus, cst: cortical spinal tract, fmaj: posterior corpus callosal bundle, ilf: inferior longitudinal tract, slfp: superior longitudinal fasciculus posterior portion, slft: superior longitudinal fasciculus temporal portion .

Multicompartmental diffusion in those fibers consistently showed signs of weakened WM integrity (lowered neurite density and enhanced OD). Most significant group differences were highlighted in medial portion of the brain in OD measures (corrected P<0.01). Micro-diffusivity revealed elevated pattern in GWI, which was consistent to our previous findings in the animal model of GWI to study neuroinflammatory response. Conversely, DTI measures showed either strengthened (higher FA, lower RD or lower MD) or opposite patterns in selective major WM tracts.

3B. GWI veterans have microstructural alterations highlighting limbic/para-limbic structures.

Multicompartmental diffusion in GM also revealed significant group differences between GWI and controls. Most significant group difference pattern was observed in limbic/paralimbic and near regions (e.g., hippocampus, entorhinal, para-hippocampal gyrus, precuneus, fusiform and cingulate gyrus). However, in GM measures, both ND and OD revealed decreased pattern in GWI compared to the control veterans. Micro-diffusivity in GM showed slightly enhanced pattern in GWI group.

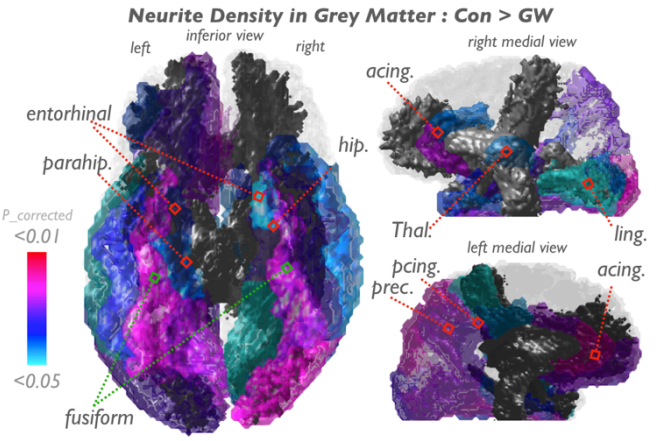

Figure 3. GM ND mapping.Con: controls, GW: Gulf war illness. Parahip: parahippocampal gyrus, pcing: posterior cingulate, prec: precuneus, acing: anterior cingulate cortex, ling: lingual gyrus.

3C. GWI veterans with mild Traumatic Brain Injury (mTBI) had more pronounced microstructural damage in limbic/paralimbic structures than GWI veterans without mTBI.

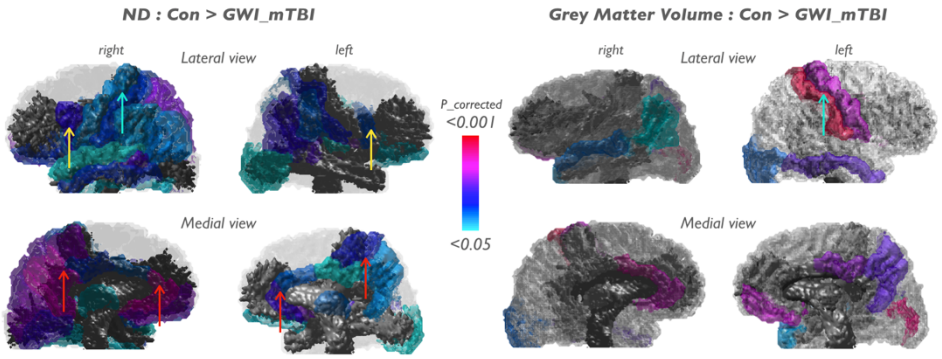

Figure 4. GWI+mTBI multicomponent diffusion (ND) and volumetry mapping. Statistically significant results are marked in color-coded structures (see color bar). Light blue arrow: pre/post central gyrus, yellow: lateral prefrontal cortex, red: cingulate/precuneus.

We found that GW veterans with mTBI (GWI+mTBI) showed more alterations than GW veterans without mTBI in microscale diffusivity parameters in most of the WM tracts and in the limbic/paralimbic regions in the cortex. GWI+mTBI subgroup showed widespread lowered patterns of ND/OD for both GM and WM. Most major WM tracts were highlighted with decreased ND/OD compared to controls. In GM, some of the most significant results were seen in bilateral precuneus (red arrow in the figure 4), bilateral rostral anterior cingulate (red arrow in the figure 4), bilateral posterior cingulate, bilateral thalamus and bilateral putamen in the GWI+mTBI subgroup. Within the GWI+mTBI subgroup, ND index captured more brain regions with significant group differences than the OD index for both WM and GM.

Micro-D results between the two subgroups were observed as the GWI+mTBI subgroup highlighted more regions with increased micro-D in GM compared to controls. The most significant results overlapped to the ND/OD results. The GWI+mTBI subgroup revealed significant group differences in additional regions in the right amygdala, the right entorhinal and the right paracentral gyrus.

The morphometry analysis did not show widespread group differences as multicomponent diffusion measures did. However, it revealed significantly reduced GM volumes in GWI+mTBI compared to controls, with the pre/postcentral being the most consistent region.

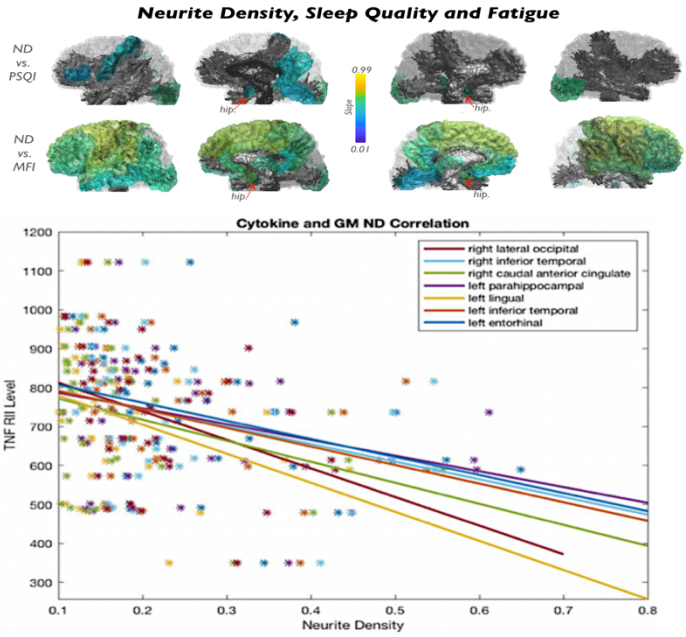

Figure 5. multicomponent diffusion, symptoms and blood cytokine relationships. Upper panel: symptoms vs imaging, Lower panel: imaging-blood cytokine relationships.

3D. Imaging measures in GWI veterans with mTBI correlated with sleep disturbance, fatigue symptoms and blood cytokine measures.

In GWI+mTBI group, we found a negative relationship between GM alterations and self-reported symptom scores (i.e., lowered micro diffusion measures correlated with more severe symptoms). Microstructural integrity (figure 5) was associated with more debilitating symptoms including pain, fatigue (MFI) and reduced sleep quality (Pittsburg Sleep Quality Index). Furthermore, alterations in limbic/para-limbic structures were also negatively correlated with proinflammatory blood cytokines (figure 5, graph), suggesting that enhanced peripheral inflammatory responses in veterans with GWI may be associated with a hindered microstructural diffusivity (indicating corresponding neuroinflammation).

<u>3E. Classifying GWI vs Control: classification performance was improved in multi-modal feature set combining DTI, microstructural diffusion and morphometrical measures.</u>

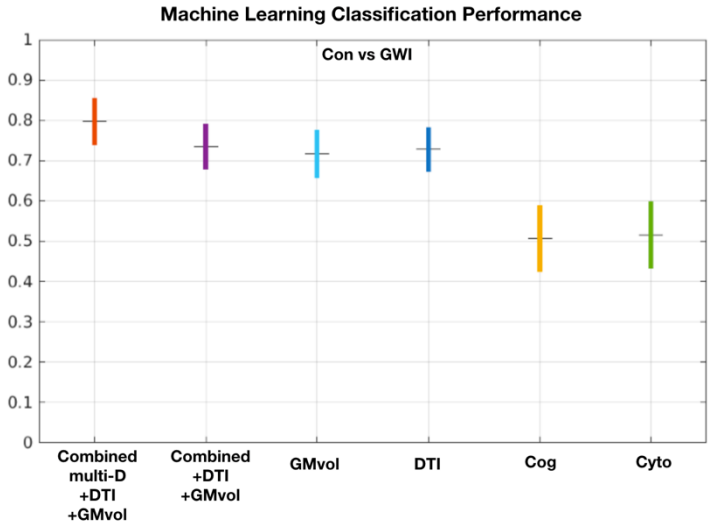

Figure 6. ML classification benchmark testing. Multi-D: ND/OD, GMvol: GM volumetry, Cog: cognitive test scores, Cyto: blood cytokine measures.

In the previous project year, a prototype of the multimodal machine learning classifier (RDF based) was tested in 12 controls and 40 GWI cases. The highest classification performance was confirmed in the multimodal combined features (WM ND + Cytokine) with 79% accuracy level (sensitivity 84%).

In this project year (year 2), we added GM micro-diffusivity measures and a new machine learning classifier (RDF-CCF). Classification tests were performed based on 91 subjects (16 controls and 76 GWI cases). Among these dataset, 13 to 15 subjects in each group were randomly subsampled in 200 times for training the classifier and rests were used as test dataset for calculating performances.

In this 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.

For classifying GWI vs controls based on the combined imaging features, following imaging features were repeatedly selected as significant contributors for the classification: left rostral anterior cingulate cortex (ND), left insula (ND), left caudal anterior cingulate (volume), left medial orbitofrontal cortex (volume), precuneus (OD), cuneus (volume), and left entorhinal cortex (volume). Interestingly, GM measures had slightly higher contributions to the classifier than the WM measures.

Combination of DTI and GM volume measures provided 74% accuracy followed by GM volume (72%) only or DTI only (73%) classifiers. These DTI or GM volume only classifier performances were up to 10% improvement compared to the previous project year results. Worst classification performances were confirmed in cognitive (~50%) and cytokine (52%) feature based classifiers. Combining cognitive or cytokine markers to the imaging markers did not help improving the classification performances (80%).

Again, neuroimaging data collection has not yet to reached the planned sample size. We will continue to add data and test classification performances in the larger data samples as they are obtained.

3F. Exploring GWI within group variances: higher Kansas subdomain scores, better classifications.

In the mass-univariate statistical analyses, we have confirmed that multidimensional diffusion measures (ND, OD, and microD) in selected cortical regions significantly correlated with fatigue, pain and neurological domain in Kansas criteria for GWI. Considering this, we have tested classification performances on the subjects with higher score (i.e., severe symptoms) in each of those Kansas domain measures. We designed iterative subject selection based on varying thresholds on each of Kansas domain scores. Training and testing of the classifier was applied on each selected subject groups. In the Kansas neurological domain (purple bar in Figure 7), this classifier provided 4% improvement (84%) in classification performance when GWI subject who had higher than 20 Kansas neuro-domain score and control subject who had lower than 10 in the same domain score were selected for the classification. Also, in GWI subjects with Kansas fatigue score (light blue bar in Figure 7) greater than 2 compared to controls, 83% classification performance was confirmed. Defining subdomain specific thresholds may improve the diagnostic value of Kansas measures.

3G. Exploring GWI within group variances II: PTSD, depression or mTBI.

We also tested classification performances on GWI subgroups based on other symptoms. Improved classification performance was confirmed from GWI veterans with depression symptoms in 10% improvement (91%) compared to whole GWI group classification (Figure 7, blue bar). Classifying GWI with PTSD (yellow vertical bar in Figure 7) symptoms also revealed 91% accuracy (over 95% accuracy in 20% classification attempts) in its classification. We also confirmed 9% classification improvement in GWI with high blood pressure (HBP, Green bar in Figure 7). GWI with more than 2 incidents on mTBI at site also revealed 9% improvement compared to the whole GWI classification.

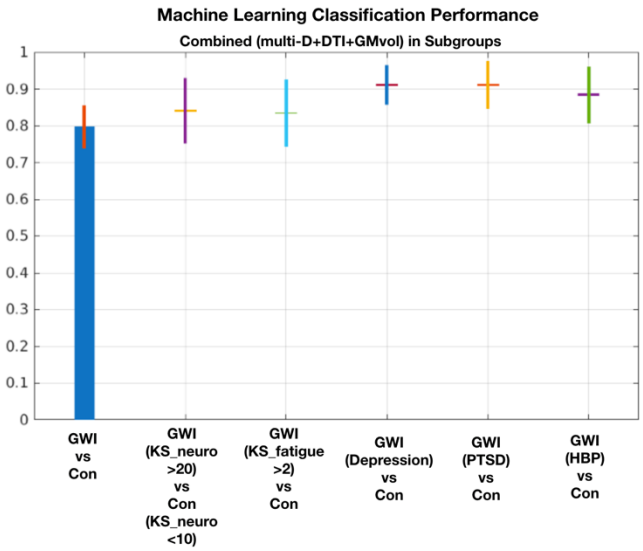

Figure 7. ML benchmark testing in different GWI subgroups. KS_neuro: Kansas neuro domain score, KS_fatigue:Kansas fatigue domain score, PTSD: post-traumatic stress disorder, HBP: high blood pressure

In other words, this also indicates that neurological deterioration can be diagnosed with 90% accuracy level based on a combination of Kansas criteria measures and other symptom profiles of GW veterans. We will continue to investigate this issue with larger sample sets in the next project year.

4. Other achievements:

- As we previously stated, neuroimaging data collection has still yet to reached the planned sample size (92/175 from Boston site, 50/75 from Texas site). Boston gulf war illness consortium (GWIC,

PI: Dr.Sullivan) will have 1 year no-cost extension to finalize this data collection. We will continue to process the newly collected data and update the analysis/classification results.

- The research team has started initial discussions on building software with graphical user interface. The software will contain all the analyses results with 3D visualization option and classification functionality for classifying newly obtained data. Three research assistants are assigned to this project. We developed initial version of 3D viewer interface and will continue to develop the software in the project year 3.

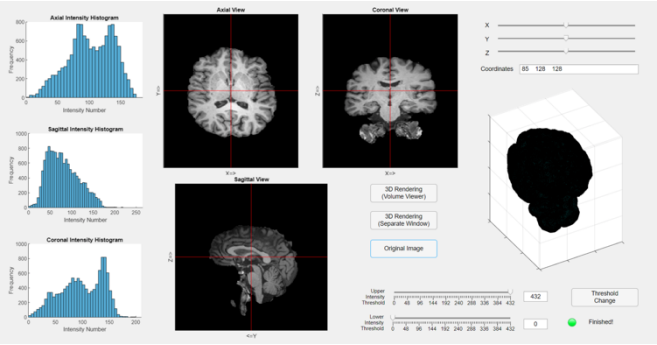

Figure 8. GUI software preparation work.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

-PI attended an international conference (International Neuropsychological Society) in Feb 21-23rd 2019.
  a.Research Assistant (Ms.Cheng) presented a poster at the meeting (Cheng et al., attached).
  b.PI, Dr.Koo, had a meeting with Drs.Linda Chao (UCSF) and Sullivan (BU SPH) at the meeting and discuss about 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) in 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 about potential treatment methods and imaging based validations.
-Three research assistants have been working in this project. PI provided trainings on computer programming and neuroimage processing. PI also provided one-to-one mentorship on RAs in the weekly meeting.
-PI attended 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 discuss about the GWI animal model study.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

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.

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

-We will continue to update subject data and work on building the classifiers combining imaging and non-imaging dataset.

-From more larger dataset, which will be continuously added in next project period, we will validate whole GWI and subgroup classification results. We will test subgroup classification performance based on pair-wise combining of different symptoms.

-We will continue to explore new imaging markers which can be extracted from existing GWIC dataset to enhance the classification outcomes.

a. Resting functional MRI data and Cerebral blood flow data will be evaluated in the next year (see section 5, actual problems). We currently finalized developing processing pipeline for these datasets.
b. We are testing individualized morphological covariance mapping to test whether those structural network measures could improve GWI classification performances. Those measures could be defined from structural MRI scans.

- We will continue to develop smart database software containing all the results achieved from this project.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

-Multidimensional diffusion MRI mapping revealed that veterans with GWI have clear group specific microstructural profiles in limbic/paralimbic regions compared to GW veteran controls. 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 veterans data, results support that microscopic diffusivity fingerprints chronic inflammation in GWI.

-Deterioration in one component of the brain can be involved in multi-symptom illness and therefore, it is expected that improved classification of GWI can be obtained by adding independent information. While DTI and volumetric measures provide information on macroscopic structural integrity of brain, micro-diffusion mapping provided meaningful supplementary information for the classification of GWI.

-Imaging measures in the paralimbic and near paralimbic regions including anterior cingulate, entorhinal cortex and precuneus had important role in improving classification performances.

-On the other hand, as we described in section 3D, cognitive or blood cytokine markers might contain information overlapped to the imaging measures. However, these measures had lower sensitivity for describing subject variances than imaging measures.

-Classification performances achieved in this work indicate that the Kansas criteria has been an effective screening method for the GWI. CDC criteria has less discriminative power on classifying GWI compared to the Kansas criteria. This needs to be further validated in a larger sample set.

-We found that GW veterans with mTBI showed more alterations than GW veterans without mTBI in microscale diffusivity parameters in most of the WM tracts and in the limbic/paralimbic regions in the cortex. We then found a negative relationship between GM alterations and self-reported symptom scores (i.e., lowered micro diffusion measures correlated to more severe symptoms). Microstructural integrity was associated with more debilitating symptoms including pain, fatigue and reduced sleep quality. 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 with only GW-relevant exposure without mTBI.

- Our results also showed that self-report depression or PTSD symptom profiles have strong diagnostic values when they combined to Kansas criteria for classifying neurological deteriorations in GW veterans.

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

-Building neuroimaging classifiers on GWI subgroups can provide more efficient classification framework then classifying the whole group.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

-Although more concrete finding can be made by the acquisition of the full samples, results indicate that illness symptoms in GW veterans mediates the chronic neuroinflammation which can be fingerprinted by microstructural imaging in limbic/para-limbic 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 be also 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. We shared our findings with Dr.O'Callaghan at CDC to plan collaborative research on this topic based on animal model of GWI and mTBI. We will apply the same imaging measurements and comparing those with immunohisotochemistry (IHC) assessments to explore this topic further.

- It is possible that layering a mTBI incident over other Alzheimer's disease (AD) risk factors (e.g., genetic or other health risks) may result in increased detrimental effects to the veteran's brain and result in AD progression. We are planning to apply multimodal imaging and classification framework to explore this issue.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

- Due to the invasive protocol to collect CSF cytokine, GWIC has been able to collect CSF markers from 7 subjects only. Although Boston GWIC will keep trying to collect those measures, PI is also planning to use alternative markers for central immune measures.

a. PI initiated discussion with Dr. Marco Loggia (Martinos Center for Biomedical Imaging, Harvard Medical School, Boston MA) regarding the translocator protein positron emission tomography (TSPO-PET) imaging. TSPO-PET has been a innovative tool to imaging glial activations in the brain in-vivo. Dr.Loggia's team has collected TSPO-PET imaging and diffusion MRI from GWI and control veterans. PI invited Dr.Loggia to PI's department to give a talk about this method and discuss about sharing data and mapping method to validate diffusion markers based on glial activation map quantified from TSPO-PET. This could be alternative but probably more targeted validation approach than combining CSF measures.

b. Chronic neuroinflammation can be associated with changes in microvasculature and also alter functional connectivity. Adding resting state functional MRI and arterial spin labeling MRI may fingerprint neuroinflammation in terms of either microvascular or functional network change in the brain. In Boston GWIC, those scans were also collected from the veterans. PI is planning to combine and compare those imaging measures to the existing data to validate our findings.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

-PI, Dr.Koo's salary was adjusted to 25% in June 2019. This is to hire additional part-time research assistant.

-In the project year 1, grant account set up was delayed and there were delay in paying co-Investigator's salary. Drs.Heeren and Killiany's unpaid salary was projected and paid through the project year 2. We are still having delays on paying Dr.Sullivan's salary. We are working with Dr.Sullivan's department to set up salary projections for paying her salary.

-PI recruited 2 more undergraduate level research assistants from biomedical engineering and neuroscience department. PI also hired a part-time graduate level research assistant from his department. We initially planned to hire 2 graduate level research assistants in 50% time of effort. However, during the project year 2, we figured out that hiring more undergraduate research assistants with strong computational and image processing background could enhance our research performances. We currently have 2 undergraduate level and 1 graduate level research assistants. All 3 assistants are part-time and it does not hurt our planned salary expenditures.

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

1. Conference poster presentation: Cheng J, Little D, Steele L, Heeren T, Killiany R, Sullinvan K, Koo B, Preliminary Evaluation of Diffusion Imaging Features for Classifying Veterans with Gulf War Illness. International Neurospsychological Society. 2019
2. Conference poster presentation: 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
3. Conference talk: 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 2019.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

-The research team has developed a pipeline for defining novel neuroimaging biomarkers and also designed classification framework for GWI based on those markers. In the next project year, this technology will be further validated using larger data samples and packaged into the software package with 3D visualization function.

-The research team proposed that combing of Kansas measures with other subjective (self-report) symptoms can be an effective marker for predicting neurological damage in GWI without performing MRI scans. Although further validation is needed, this approach could be an easy to use prescreening diagnostic tool.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name:	*Bang-Bon Koo*
Project Role:	*Principal Investigator / No Change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	*Kimberly Sullivan*
Project Role:	*Co-investigator / No Change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	*Ron Killiany*
Project Role:	*Co-investigator / No Change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	*Timothy Heeren*
Project Role:	*Co-investigator / No Change*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	
Contribution to Project:	
Funding Support:	

Name:	*Jasmine Cheng*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Ms. Cheng has been working on software programming, data processing and organizing the imaging measurement outcomes.*
Funding Support:	

Name:	*Wendy Guo*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Ms. Guo has been working on data processing and organizing the imaging measurement outcomes.*
Funding Support:	*Start supporting on Nov 2018*

Name:	*Alekha Kolli*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Ms. Kolli performed work on software programming.*
Funding Support:	*Supported from this grant. Ended on April 2019.*

Name:	*Guan Yi*
Project Role:	*Research Assistant (Graduate Student)*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	*Ms. Yi performed work on working on data processing and programming.*
Funding Support:	*Start 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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Principal Investigator (Dr.Bang-Bon Koo):
Dr.Koo has an additional funding support for his co-investigator role in the following grant.
Title: Boston Biorepository, Recruitment and Innovative Network (BBRAIN) for GWI
Suporting Agency: Department of Defense (CDMRP/GWIRP GW170055)
P.I.: PI: Sullivan
Duration: 9/01/18 – 8/31/21
Effort: 0.60

Co-investigator (Dr.Killiany):
Dr.Killiany has an additional funding support for his co-investigator role in the following grants.
Title: Boston Biorepository, Recruitment and Innovative Network (BBRAIN) for GWI
Suporting Agency: Department of Defense (CDMRP/GWIRP GW170055)
P.I.: PI: Sullivan
Duration: 9/01/18 – 8/31/21
Effort: 0.60

Title: The Gulf War Illness Clinical Trials and Interventions Consortium (GWICTIC)
Suporting Agency: Department of Defense (CDMRP/GWIRP GW170055)
P.I.: PI:Klimas
Duration: 9/01/18 – 8/31/22
Effort: 0.60

Co-investigator (Dr.Sullivan):
Dr.Sullivan has an additional funding support for her PI and co-investigator role in the following grants.
Title: Boston Biorepository, Recruitment and Innovative Network (BBRAIN) for GWI
Suporting Agency: Department of Defense (CDMRP/GWIRP GW170055)
PI: Sullivan
Duration: 9/01/18 – 8/31/21
Effort: 2.4

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
- 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. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

Reference cited:

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. http://doi.org/10.1016/j.bbi.2017.08.003

Koo, B.-B., Hua, N., Choi, C.-H., Ronen, I., Lee, J.-M., & Kim, D.-S. (2009). A framework to analyze partial volume effect on gray matter mean diffusivity measurements. *NeuroImage*, *44*(1), 136–144. http://doi.org/10.1016/j.neuroimage.2008.07.064

Koo, B.-B., Choi, K., Ronen, I., Lee, J.-M., & Kim, D.-S. (2010). Quantitative mapping of diffusion characteristics under the cortical surface. *Magnetic Resonance Imaging*, *28*(8), 1175–1182. http://doi.org/10.1016/j.mri.2010.03.020

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

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.

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.

Conference abstract:
Cheng J, Little D, Steele L, Heeren T, Killiany R, Sullinvan K, Koo B, Preliminary Evaluation of Diffusion Imaging Features for Classifying Veterans with Gulf War Illness. International Neurospsychological Society. 2019

PAPER OR POSTER ABSTRACT SUBMISSION DETAILS:

Date Submitted (still in DRAFT if blank): August 9, 2018, 8:16 PM

Control ID: 3055998

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

Preferred Presentation Type: Paper or Poster

Selected Category: Neuroimaging

Selected Keyword(s): neuroimaging: structural connectivity, traumatic brain injury, neurotoxicity.

Contact / Submitting Author: Bang-Bon Koo

Presenter (underlined in list below): Jasmine Cheng

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ABSTRACT PROOF--PLEASE REVIEW CAREFULLY:

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

AUTHOR(S): J. Cheng[1], D. Little[2], L. Steele[2], T. Heeren[4], R. Killiany[3], K. Sullivan[4], B. Koo[1]

Affiliations: J. Cheng, B. Koo, Anatomy and Neurobiology, Boston University School of Medicine, Boston, Massachusetts, UNITED STATES;
D. Little, L. Steele, Baylor College of Medicine, Houston, Texas, UNITED STATES;
R. Killiany, Boston University School of Medicine, Boston, Massachusetts, UNITED STATES;
T. Heeren, K. Sullivan, Boston University School of Public Health, Boston, Massachusetts, UNITED STATES;

ABSTRACT BODY:

Objective : We assessed brain diffusion MRI data from the first 32 veterans enrolled in the ongoing Gulf War Illness (GWI) Consortium study to disentangle different components of intra-voxel diffusion to assess brain imaging features specific to GWI.

Participants and Methods: MRI data from 32 GW veterans (11 GWI cases without mild TBI, 12 GWI cases with mTBI during the war, 9 GW veteran controls) were evaluated. Diffusion imaging were collected based on 124 directions with multiple b-vals and reconstructed by tensor (DTI) and multicompartmental modeling. Major white matter (WM) fibers were defined based on probabilistic tracking and applied sampling diffusion measures. Extracted tract measures were applied for testing group differences on the following pairs: 1) all_GWI cases vs controls, 2) GWI with mTBI vs controls, 3) GWI without mTBI vs controls, 4) GWI with mTBI vs GWI without mTBI. We applied multi-layered decision forest maps on the diffusion measures. Each classifier performance was tested based on a leave one out cross validation.

Results : DTI measures showed either strengthened (higher FA, lower RD or lower MD) or opposite patterns in major WM tracts in GWI cases. Multicompartmental diffusion in those fibers consistently showed signs of reduced WM integrity (lowered ICVF and enhanced OD). In the subgroup analyses, GWI cases with mTBI showed more pronounced WM alterations than GWI cases without mTBI. Overall, the highest classification performance for identifying GWI cases was confirmed in the multicompartmental model (accuracy:88%) followed by DTI (62%).

Conclusions : Multi-compartmental diffusion modeling results confirmed that the complex DTI patterns were consistent with degenerative patterns and appear to hold promise for differentiating GWI cases from controls. Combining machine-learning technology with multi-compartmental diffusion measures may allow for better classification of GWI cases and may also provide a better understanding of the complex pathobiology and potential subgroupings of GWI.

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Contact Email for Publication: bbkoo@bu.edu

Contact Author's Listed Institution: Boston University School of Medicine

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

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

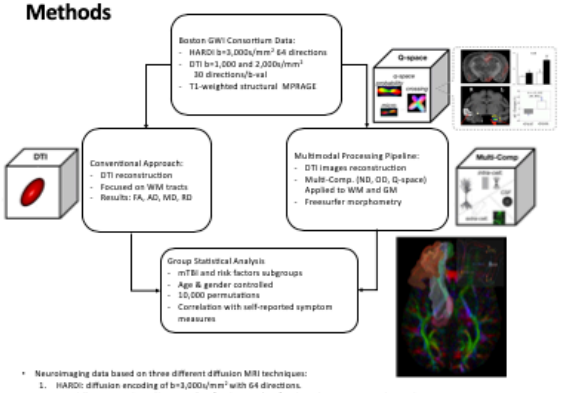
Participants

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/mm² with 64 directions.
  2. DKI: diffusion encoding of b=1,000s/mm² and 2,000s/mm² 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

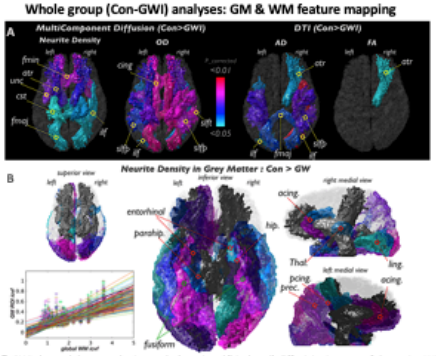


Figure 1. Corrected P value mapped on a color spectrum, red representing p values less than 0.01 and light blue as 0.05.
(A) Reconstruction on white matter tracts with significant p values from both Neurite Density, orientation dispersion (OD) and DTI (right part). Figure shows group comparison results 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 Colossal tract (fmin) & uncinate fasciculus.*
  - *OD: : Highest group difference in anterior/posterior Colossal 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	Fmincr (ND & OD)	Fmincr	Bilat_cau_aCing	R_cau_aCing	Bilat_cau_aCing	
	Bilat_slft (OD)	Bilat_slfr	Bilat_rost_aCing (ND & OD)	L_rost_aCing	Bilat_supra_marginal (vol)	
	R_ilf (ND & OD)	Bilat_ilf	Bilat_fusiform (ND)	Bilat_fusiform (vol)	Bilat_postcent (vol)	
	Bilat_slfp (OD)	R_slfp	R_hippocampus (ND & OD)	R_hippocampus	Bilat_middle_temp (vol)	
	R_str (ND & OD)	R_str	R_entorhinal (ND)	R_entorhinal	R_trans_temp (vol)	
Control - GWI with mTBI with risks	Bilat_unc (ND)	L_unc	Bilat_rost_aCing (ND)	L_rost_aCing	R_fusiform (vol)	
	Bilat_ilf (ND)	R_ilf	Bilat_post_cing (ND)	Bilat_post_cing	R_parahipp (vol)	
	Fmincr (ND)	Fmincr	R_paracentral (ND)	R_paracentral	No sig.	
	Bilat_str (ND)	Bilat_str	R_banks_sts (ND)	R_banks_sts	No sig.	
	Bilat_cst (ND)	R_cst	R_supra_marginal (ND)	R_supra_marginal	No sig.	
Control - GWI without mTBI with risk	Bilat_str (ND)	L_str	R_lat_occipital (OD)	R_lat_occip	Bilat_lat_occip (vol)	
	Fmincr (ND)	R_ilf	L_rost_aCing (ND)	R_post_cing	L_transverse_temp (thi)	
	Bilat_cst (ND)	No sig.	L_cuneus (ND)	R_inf_parietal	No sig.	
	No sig.	No sig.	L_temp_pole (OD)	R_fusiform	No sig.	
	No sig.	No sig.	L_isthmus_cing (OD)	R_banks_sts	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, lef: 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).*
</supplementary>

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

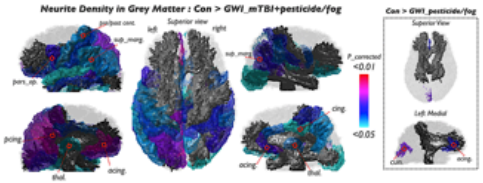


Figure 2. 3D brain mapping of significant statistical results from mTBI subgroup ND processing. Corrected P value displayed on a color spectrum, red representing p values less than 0.01 and light blue as 0.05. Exposure to either one or both risk factors (pesticide or fogged) were both taken into account.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

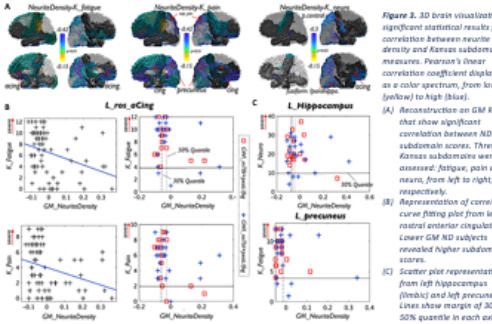


Figure 3. 3D brain visualization of significant statistical results from correlation between neurite density and Kansas subdomain measures. Pearson's linear correlation coefficient displayed as a color spectrum, from low (yellow) to high (blue).
(A) Reconstruction on GM ROIs that show significant correlation 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)

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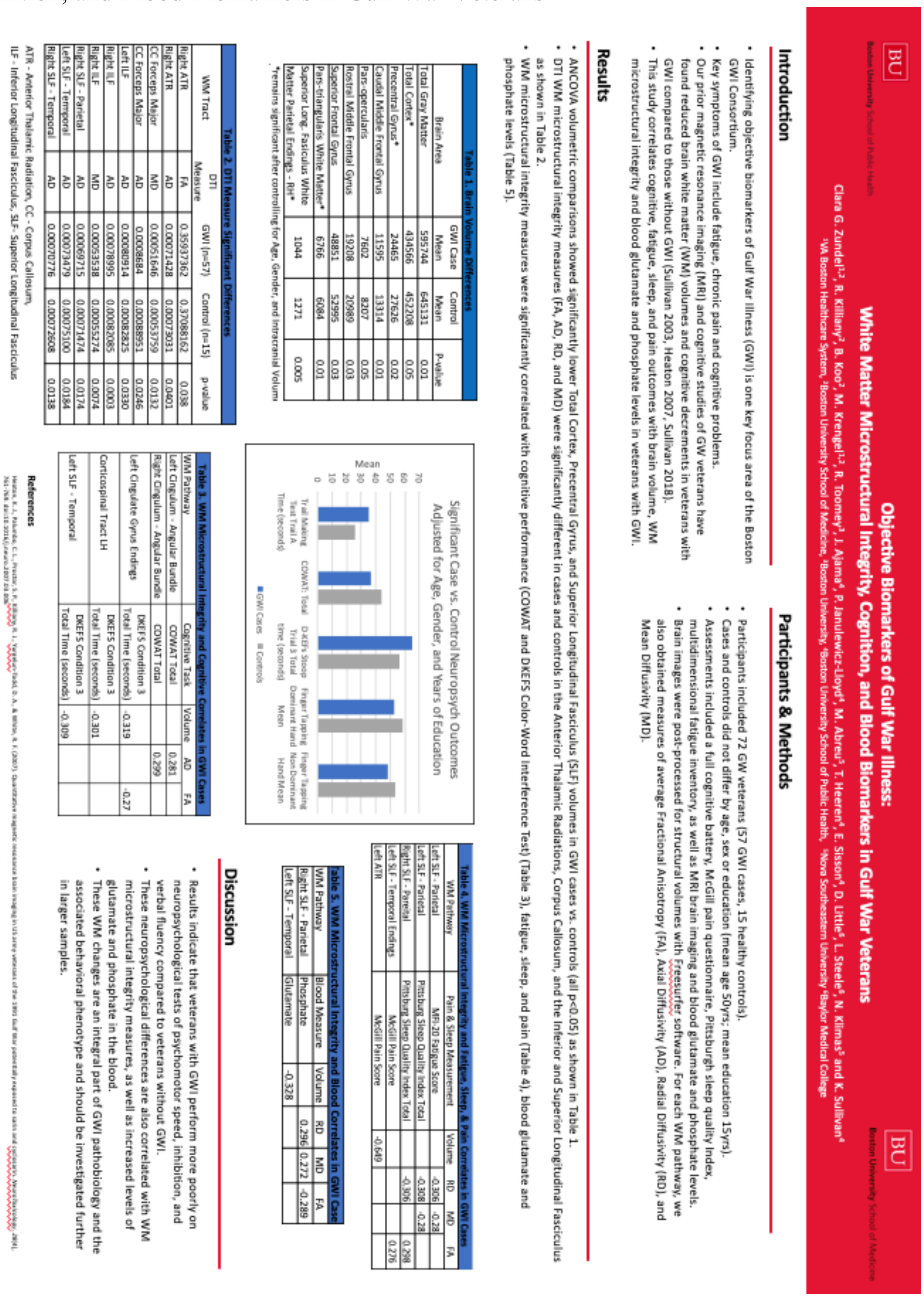
1. O'Donovan et al. (2013). Altered inflammatory activity associated with reduced hippocampal volume and more severe posttraumatic stress symptoms in Gulf War veterans. Psychoneuroendocrinology, 38, 2059–2066.
2. Abhinav et al. (2006). Impaired immune function in Gulf War illness. BMC Medical Genomics, 2(1), 12.
3. Chao et al. (2012). Effects of low-level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. Neuroimage, 59, 887–900.
4. Chao et al. (2011). Effects of low-level sarin and cyclosarin exposure on white matter integrity in Gulf War Veterans. Neurotoxicology, 32, 1087–1095.
5. Koo et al. (2016). Long-term effects of curcumin in the rat human primate brain. Brain Research Bulletin, 122, 48–54.
6. Koo et al. (2016). Corticosterone potentiates LPS-induced neuroinflammation and affects high-order diffusion imaging in a rat model of Gulf War Illness. Brain, Behavior, and Immunity, 57, 42–56.
7. Barnes et al. (2016). Association of Mild Traumatic Brain Injury With and Without Loss of Consciousness With Dementia in US Military Veterans. JAMA Neurol., 73(9):1055–1062. doi:10.1001/jamaneurol.2016.1693

Conference Poster:

Conference poster presentation: 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



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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. Fasciculus White Matter Parietal Endings - RH*	1044	1271	0.005

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

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 - Temporal	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

Significant Case vs. Control Neuropsych Outcomes Adjusted for Age, Gender, and Years of Education

Mean
70
60
50
40
30
20
10
0
Trail Making Test Trail A Time (seconds)
COWAT: Total
D-KEFs Stroop Trial 3 Total time (seconds)
Finger Tapping Dominant Hand Mean
Finger Tapping Non Dominant Hand Mean
GWI Cases
Controls

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		

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 Case

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

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

Funding: This study was funded by CDMRP award # GW120037

Conference talk: 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 2019.

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

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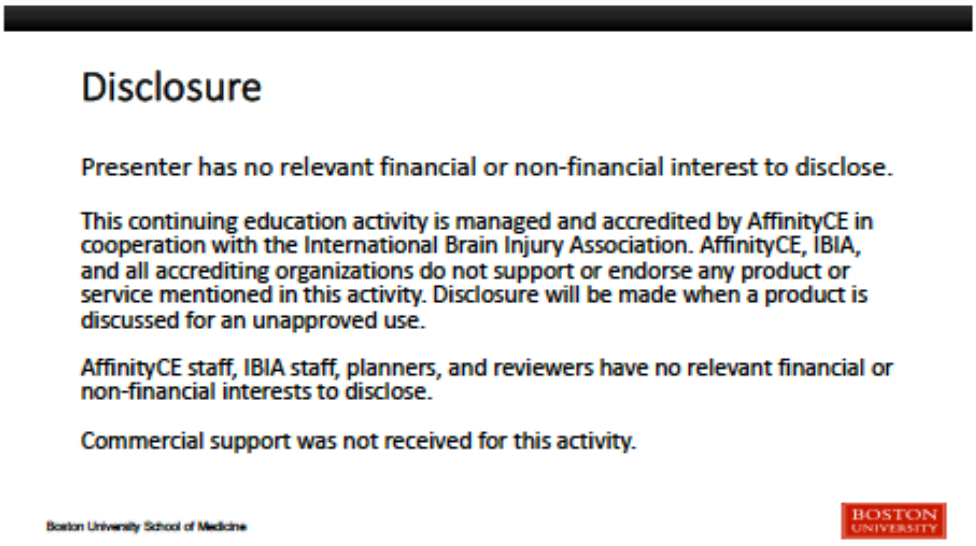

Learning Outcomes

- 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.

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Overview

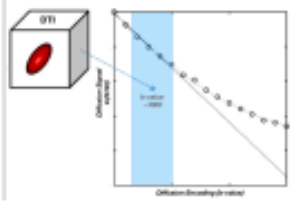
- Neuroimaging observations in 1991 Gulf War (GW) Veterans with a history of mild Traumatic Brain Injury (mTBI).
- Relationships to Illness Symptoms
- Relationships to Blood immune markers

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/mm² 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.

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

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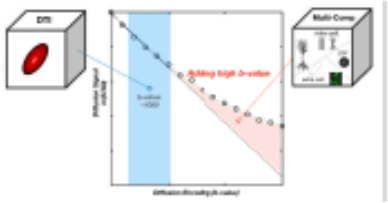

standard measure for studying tomy and connections.

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

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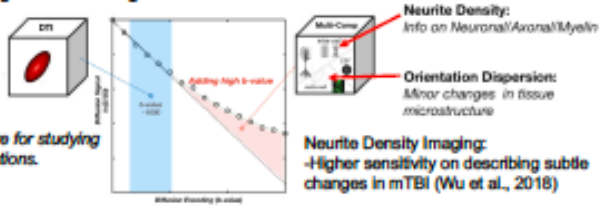

-DTI has been a standard measure for studying white matter anatomy and connections.

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 & GM/WM Microstructural Alterations in 1991 Gulf War Veterans

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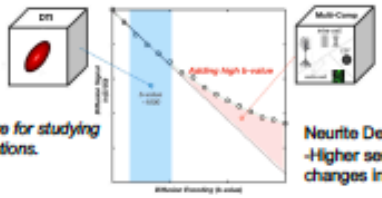

-DTI has been a standard measure for studying white matter anatomy and connections.

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.

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Participants

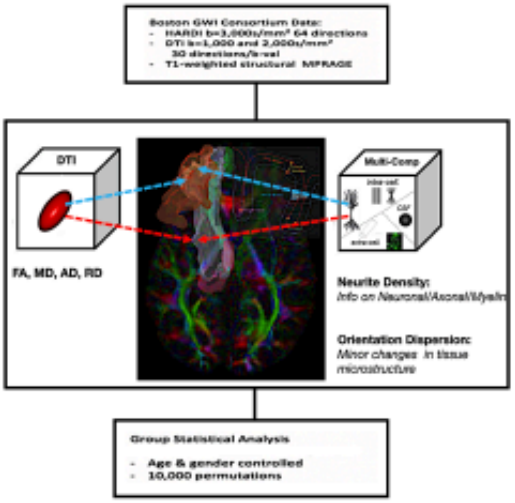
Table 1. Participants Demographics		
	GWIC veterans (n=68)	Mean age
Healthy Controls (GW veterans)	15	53.58
Veterans with GWI	53	51.17
	GWI Subgroups	
mTBI during war (labeled: mTBI)	20	52.53
Without mTBI (labeled: noTBI)	33	51.25

- 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 & GM/WM 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).



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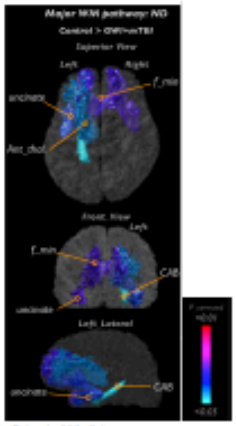
Results:

Multi-component diffusion imaging in WM

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

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

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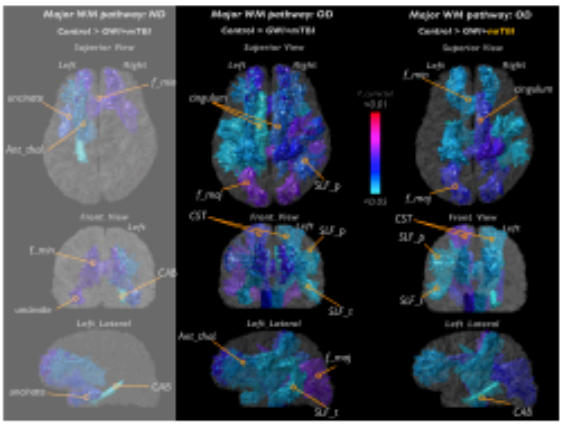
Results: *Multi-component diffusion model (White Matter Connections)*



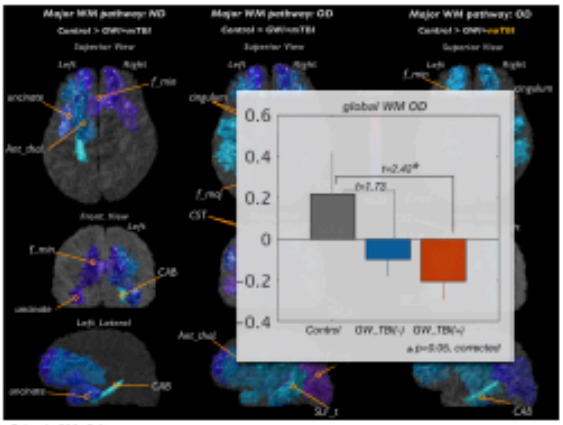
Lowered Orientation Dispersion in both GWI +mTBI and -noTBI groups.

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.

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

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



Global WM OD showed clear distinction between groups.

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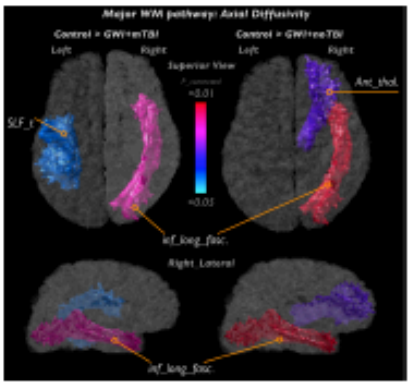
Results: *Diffusion Tensor Model*

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.



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

Results:

Multi-component diffusion imaging in GM

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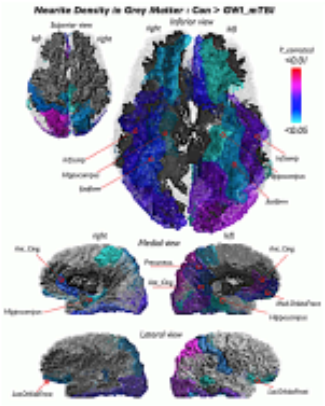
Results: *Multi-component diffusion model in the Gray Matter*



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.

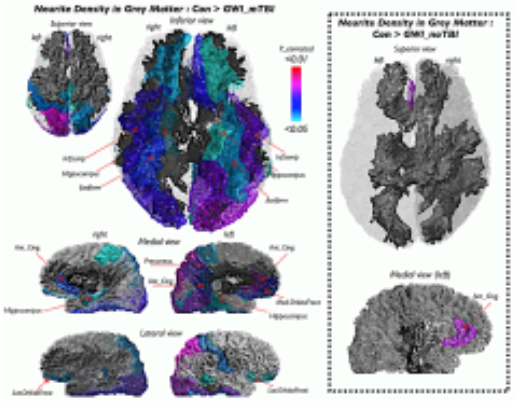
- *Cingulate*
- *Hippocampus*

And low Neurite Density regions connected to the limbic structures

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

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

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.

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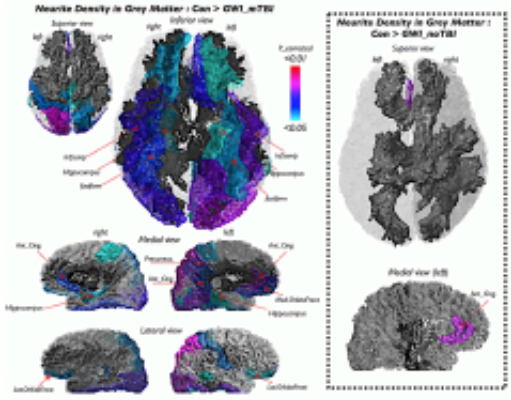
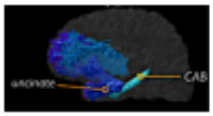
Results: *Multi-component diffusion model in the Gray Matter*



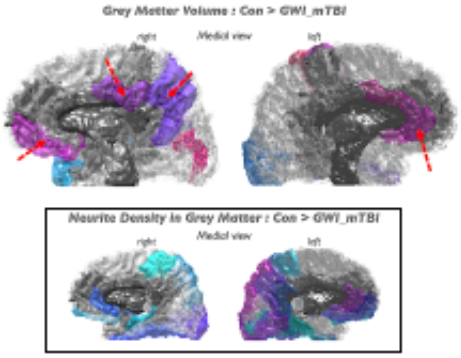
Taken together with the WM Neurite Density results,



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

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

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.*

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Results:

Imaging, Illness Symptoms and Blood Immune Markers

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Results: *Imaging and Symptoms*

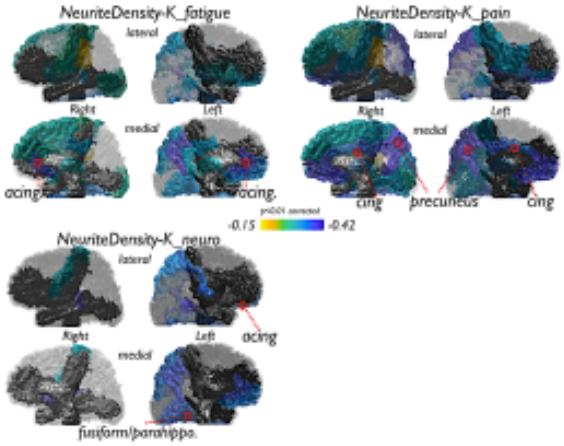
-GM Neurite Density measures had mild-to-moderate negative correlations to some of the Kansas sub-domain scores:

- *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.



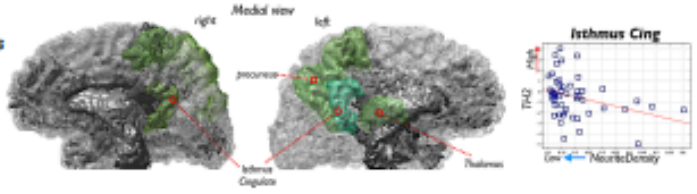
Results: *Imaging and Peripheral Immune measures*

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

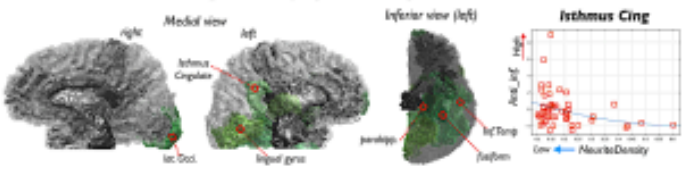
-> lowered Neurite Density was related to the elevated TH2 and Anti- inflammatory cytokine markers

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

GM Neurite Density vs TH2 Cytokine Group



GM Neurite Density vs Anti-inf. Cytokine Group



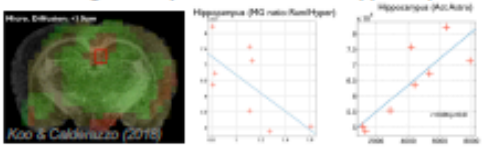
Mild TBI & GM/WM Microstructural Alterations in 1991 Gulf War Veterans

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.

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

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/anatneuro/research/bio-imaging-informatics-lab/)

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

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Thank you.

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