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14. ABSTRACT

In this first year grant period, we developed an image processing pipeline for multi-parametric diffusion magnetic resonance imaging data, which has been collected from the Gulf War Illness Consortium. The pipeline was designed to apply the most up-to-date diffusion reconstruction techniques to provide multi-compartmental diffusion model based measures, q-space reconstructions as well as diffusion tensor metrics. As we planned and mentioned in the statement of work, major milestone in the year 1 of this grant was performing diffusion imaging feature extractions from the image data. As such, we processed data from 73 veterans for extracting major white matter bundles, a basis for cortical network reconstructions. In addition, we added gray matter diffusivity mapping to investigate potential microstructure changes in the cortex. Since the other milestone for year 1 to the middle of the year 2 period was building unimodal classifier of GWI neuroimaging and neuroimmune data, we started building unimodal classifiers on each features. Most significant findings in this project period was that the best classification results were observed in a classifier based on the advanced diffusion imaging measures. This may indicate that the advanced diffusion imaging measures might describe the underlying pathophysiology of GWI better than other markers. However, this work needs to be continued to the year 2 period to have clearer figures.

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

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Table of Contents

1. INTRODUCTION:

Symptoms of Gulf War illness (GWI) typically include persistent headaches, widespread pain, fatigue, memory and concentration problems and other difficulties. Converging evidence suggests that a clear understanding of brain-immune interactions can help us to understand the origin of these symptoms. Biomarkers from different body systems affected in GWI including brain, immune and genetic predisposition may reflect different yet connected aspects of this multi-symptom illness. To bring novel insights and technologies for treating GWI, this project was planned to incorporate the abundant amount of biomarker data which is currently being collected by the Boston GWI consortium (GWIC). Brain connectomic measures extracted from magnetic resonance imaging (MRI), central inflammatory measures in cerebrospinal fluid, peripheral inflammatory cytokine measures in blood samples and genetic SNP measures from saliva data from GWI cases and controls are used to uncover multivariate data distribution from these different biomarkers. The primary aim is to develop a novel supervised machine learning framework based on multi-modal biological measurements to establish a single subject level diagnostic inference marker of GWI. Based on the information collected in this project, we will also build a novel user-interactive database system which provides information from the classifiers and identifies features of GWI at the individual subject level.

2. KEYWORDS:

Gulf War Illness
White matter integrity
Gray matter microstructure
Connectivity
Morphometry
Neuroinflammation
MRI marker
Machine Learning
Brain mapping
Blood Cytokine
Mild TBI
Chemical exposure
Pesticides
Kansas criteria
CDC criteria

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major tasks planned in the first project year includes obtaining Institutional Review Board (IRB) approvals and building unimodal classifiers of GWI neuroimaging data. IRB approval was obtained on Nov. 17th 2017 from the BU Institutional Review Board and HRPO approval was received from DoD on Jan. 2nd 2018. This was the first milestone to be achieved in the approved SOW. We achieved both approvals in 4 months from the project starting date.

The second major task was building a unimodal classifier of GWI based on neuroimaging data collected in the Boston GWIC. There were 3 subtasks planned in the SOW. Subtask 2-1 was planned to process diffusion data. Subtask 2-2 was planned to perform cortical modeling and network reconstruction on GWI. Both subtasks were planned to be performed from 4th to 10th project months. The 3rd subtask (2-3) was planned to build classifiers per each diffusion map during 11th project months to the half of the 2nd project year. To date, a total 83 GWIC participants imaging and biomarker data was received, data was processed and put into the computational pipeline. The 3rd major task aimed to build a unimodal classifier of GWI on neuroimmune markers during 11th to 18th project months.

What was accomplished under these goals?

Major goals and accomplishments for year 1 are summarized in the table below:

Research Specific Tasks	Timeline	Status	Re-action Plan
Major Task 1: Obtain IRB approval	**months**		
Subtask 1-1: Obtain local IRB approval	1-3	Achieved, Nov 17th,2017	-
Subtask 1-2: Obtain HRPO approval	3-4	Achieved, Jan 2nd, 2018	-
Milestone(s) Achieved: Obtained local IRB and HRPO approvals	1-4	Accomplished	-
Specific Aim 1: Building unimodal classifiers per each biological measure of GWI	**Timeline**		
Major Task 2: Build a unimodal classifier of GWI neuroimaging data	Months		
Subtask 2-1: 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	Achieved on all of the data currently collected in GWIC (total 83 subject data).	GWIC has been granted a 1 year no cost extension to continuing subject recruitment efforts. GWIC will use new recruitment efforts to complete recruitment goals including using listservs, veteran organization outreach, enhanced social media presence and veteran advisory group outreach. All newly acquired data will be promptly processed and updated into the processing pipeline
Subtask 2-2: Cortical modeling and network reconstruction - includes visual inspection and corrections (data from 250 study participants; 150 cases, 100 controls)	4-10	Achieved on all of the data currently collected in GWIC (total 83 subject data).	
Subtask 2-3: Build classifiers per each diffusion map	11-18	Initial version classifiers built	Continue to work on building classifiers
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	Achieved on existing data On-going On-going	-
Major Task 3: Build a unimodal classifier of GWI neuroimmune marker features			
Subtask 3-1: 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	Initial classifier will be tested once we have 20 samples. We'll continue to add CSF markers from GWIC enhanced recruitment efforts
Subtask 3-2: Build a classifier of blood immune markers (250 GW veterans; 150 GWI cases, 100 controls)	11-18	On-going	-
Milestone(s) Achieved: 1. classifiers and their benchmark tests (sensitivity of GWI). 2. descriptions of important features.	11-18	Preliminary results presented at group meetings	-

1) Major activities:

Major activities in this project year include the followings: 1) setting up on the computing environment setup; 2) building process pipelines for the neuroimaging data; 3) post-processing of neuroimaging data; 4) Quality assurance work on the processed results. In detail, the research team built a parallel computing environment for processing the neuroimaging data. Unix cluster computing system allows up to 180 computing cores with 2 graphic processing unit boxes which allows more than 50 times faster processing speed compared to desktop computing systems. The image processing pipeline was built and installed to the system.

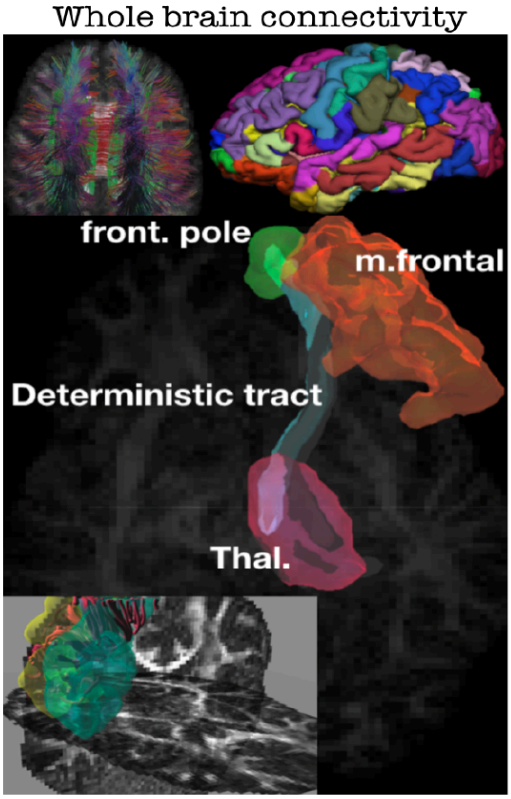

Figure 1. Illustration of the major tract reconstruction on GWI MRI data.

The pipeline performs following processing:
- Cortical surface modeling and defining regional cortical structures
- Co-registration between structural and diffusion MRI
- Diffusion data preprocessing for correcting motion and eddy current distortions
- Diffusion modeling on diffusion tensor map, q-space orientation distribution map, high order diffusion map and microscopic diffusivity map (MicroD).
- Connectivity reconstructions
- Local diffusivity mapping to tracts and cortical volumes

Based on this computing environment, we finished the cortical modeling and brain connectional network reconstruction on 83 participants imaging data. We performed repeated quality assurance reviews (i.e., detailed visual inspection, checking modeling errors, distributions and outliers in the quantification values) on the processed results. Based on these models, variant brain tissue diffusivity quantifications obtained from the subtask 2-1 were then mapped to the white matter major tracts and cortices to describe local brain structures and networks. For the subtask 2-3, initial stage classifiers and feature descriptions in 3D mapping was also performed.

For task 3, diffusion connectivity markers extracted from the task 2 were used for building initial version of the classifiers. Since the task 3 was planned to be performed from 11 months to the first half of the 2nd year, we will continue to work on building classifiers based on each imaging measures.

As we planned to build classifiers on non-imaging markers including CSF immune markers and blood immune markers starting in the end of the year 1, we started to build the initial version of the classifier using blood immune markers.

2) Specific objectives:

In the project year 1, we aimed to acquire high quality post-processed data and neuroimaging classifiers. From the processed data, we also aimed to explore whether GWI is more associated with alterations in the local brain components especially in the microscopic structural integrity and connectivity in the white matter (WM). Technically, advanced concept on multi-parametric diffusion MRI was of our interest to see whether and what extent the microscopic diffusivity could identify different stages of chronic neuroinflammation in the veteran's brain, similar to what we have confirmed from the imaging of animal models of GWI (Koo et al., 2018). A goal was to introduce quality imaging measures to the GWI research community.

3) Significant results and Key outcomes

Although neuroimaging data collection has yet to reached the planned sample size (175 from Boston site, 75 from Texas site), we performed assessments on the existing dataset. This was to preliminarily assess sensitivity of multi-parametric diffusion markers of GWI.

Diffusion imaging was based on 124 directions with 3 b value diffusion encodings (1000, 2000, 3000 s/mm^2) and reconstructed by both tensor model (DTI) and multi-compartmental modeling as previously described. Major WM fiber pathways were defined based on the probabilistic tracking method. Diffusion measures were mapped to each tract. Extracted tract measures were applied for testing group differences on following pairs: 1) all GWI vs controls, 2) GWI with mTBI vs controls, 3) GWI without mTBI vs controls, 4) GWI with vs without mTBI. We also tested multi-layered decision forest classifier on the diffusion measures. Each classifier performance was tested based on a leave one out cross validation.

Table 1. Group analysis on the tract diffusion measures. Whole group analyses are shown in the Control-GWI section followed by the subgroup analyses. Con>gwi denotes the diffusion value higher in the control group and con<gwi indicates the opposite pattern. We only listed ***the tracts with p<0.05 level differences and asterisk is used for highlight the tracts with p<0.005 level group differences***. Group assessments under different GWI criterions (Kansas measures and CDC scores) are listed separately. Intracellular diffusivity assessment based on the Kansas criterion highest sensitivity on describing group differences among all measures.

	Control – GWI	Control – GWI+TBI	Control – GWI-TBI
Intracallular diffusivity	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	fminor*, lh_atr, lh_cab, lh_ccg, lh_cst, lh_unc*, fmajor, lh_ilf, lh_slft, lh_slfp rh_ccg, rh_cst, rh_unc, rh_atr, rh_cab, rh_ilf, rh_slfp, rh_slft	fminor, lh_atr, lh_cab, lh_ccg, lh_cst, lh_ilf, lh_slfp, lh_unc, rh_atr, rh_cab, rh_ccg, rh_cst, rh_sct, rh_ilf, rh_slfp, rh_slft, rh_unc	None
CDC	fminor, rh_unc	fminor, lh_atr, lh_unc, rh_atr, rh_unc, rh_cst, rh_ilf, rh_slfp	
Intracellular Orientation Dispersion	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	rh_ccg*, rh_cab, rh_ccg*, fmajor	rh_ccg, fmajor	rh_ccg
CDC	lh_cab, lh_ilf, fmajor rh_cab*, rh_unc	fmajor, lh_cab, lh_ilf rh_cab, rh_slfp	lh_cab rh_cab
GFA	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	fmajor, fminor, lh_atr, lh_cst, lh_slfp, lh_slft, lh_unc rh_atr, rh_cst, rh_ilf, rh_slfp, rh_slft, rh_unc*	lh_atr, rh_sct, rh_slfp, rh_unc	rh_unc, rh_atr
CDC	lh_atr, lh_cst, rh_atr, rh_slft	lh_atr, lh_cst, rh_atr, rh_slft, rh_cst, rh_slfp, rh_unc	
MicroDiffusion	**con<gwi**	**con<gwi**	**con<gwi**
Kansas	fmajor rh_cab	NONE	rh_cab
DTI-Axial Diffusivity	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	fmajor, fminor, lh_ilf, lh_slfp, lh_unc rh_atr, rh_ilf**, rh_slfp*, rh_slft	fmajor, lh_ilf, lh_slft rh_ilf*, rh_slfp, rh_slft	lh_ilf, lh_slft, fminor rh_atr, rh_ilf*, rh_slfp, rh_slft
CDC	fminor*, lh_unc, rh_cst, rh_ilf, rh_slft, rh_slfp	lh_unc, lh_slfp rh_cst, rh_ilf, rh_slfp, rh_slft	fminor, lh_unc rh_cst
DTI-Radial Diffusivity	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	fmajor, lh_slft	lh_slfp, rh_atr	
CDC	lh_slft, lh_unc, rh_slfp		
DTI-Mean Diffusivity	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	fmajor rh_ilf	fmajor, rh_ilf	
CDC	lh_slft, lh_unc, rh_cst, rh_slfp, rh_slft*	fmajor, lh_cst, lh_slft, lh_unc rh_cst, rh_ilf, rh_slfp, rh_slft**	lh_sflt
FA	**con>gwi**	**con>gwi**	**con>gwi**
Kansas	rh_atr, fminr		rh_atr, fminor, lh_atr
CDC	fminor, lh_atr		

Lh_: left hemisphere, rh_:right hemisphere, cst: Corticospinal tract, ilf: Inferior longitudinal fasciculus, unc: Uncinate fasciculus, atr: Anterior thalamic radiation, ccg: Cingulum – cingulate gyrus (supracallosal) bundle, cab: Cingulum – angular (infracallosal) bundle, slfp: Superior longitudinal fasciculus – parietal bundle, slft: Superior longitudinal fasciculus – temporal bundle, fmajor: Corpus callosum – forceps major, fminor: Corpus callosum – forceps minor

From the preliminary group assessments, we confirmed consistent signs of weakened WM integrity from the high order diffusion modeling measures, such as Intracellular diffusivity, orientation dispersion. Based on 73 participants's scans (58 cases, 15 control veterans), we have found following patterns (see Table 1 for summary):

No significant differences in the tract morphology between controls and GWI

- WM tract morphometry: GWI group showed lower tract volume in the right superior longitudinal fasciculus (parietal compartment) than controls. Other WM tracts were not different between the groups.

Significant degenerative patterns in GWI captured from multidimensional diffusion markers

- Intracellular restricted diffusion component in GWI: GWI group showed significantly lower intracellular diffusivity pattern on intracellular restricted diffusion component in most of the major WM tracts including the anterior corpus callosum tract, the anterior thalamic tract, the left cingulate angular bundle, the cingulate gyral bundle, the cortical spinal tracts, the superior longitudinal fasciculus (temporal) and uncinated fasciculus. We also confirmed that GWI veterans with 'in theater' mTBI history during the war (GWI-TBI in fig1, Table 1) have more significant degenerative patterns than the GWI veterans without mTBI during the war (GWI-noTBI in fig 2) in most of the major WM connections. Lowered patterns shown in GWI may fingerprints degenerations in WM major connections.

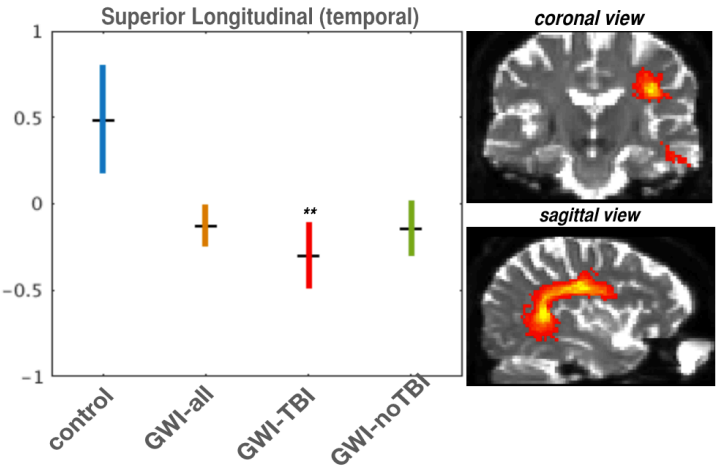

Figure 2. Intracellular diffusion mapping on the superior longitudinal tract

- Micro diffusion component: Micro diffusion measure was used for assessing the animal model of GWI to study neuroinflammatory response and revealed that elevated pattern in GWI model (Koo et al., 2018). In the human data, the GWI group also showed statistically significant higher microscale diffusivity pattern in the posterior callosal tract, right cingulum angular bundle and right cingulate gyral bundle. GWI veterans with 'in theater' mTBI history have slightly higher degenerative patterns than the GWI veterans without mTBI in both the right cingulum angular bundle and the right cingulum gyral bundle.

- Q-space imaging (GFA): The GWI cases showed statistically significant lower values than controls on q-space indices in the bilateral anterior thalamic tracts, the bilateral corticospinal tracts and the right uncinated tract. GWI cases with 'in theater' mTBI history have more significant degenerative patterns than the GWI veterans without mTBI in these tracts.

Enhanced and weakened patterns shown in DTI

- Diffusion Tensor Imaging indices (FA & RD): Although multi-dimensional diffusion indices revealed degenerative patterns, DTI measures showed either enhanced or weakened pattern in GWI. GWI group showed low fractional anisotropy (FA) in anterior callosal tract, which is a pattern of degeneration. However, opposite pattern was also confirmed posterior callosal tract of GWI veterans with 'in theater' mTBI history compared to GWI without mTBI group. GWI group showed lower axial diffusivity compared to controls in the callosal tracts, bilateral inferior longitudinal tracts, bilateral superior longitudinal tracts, left uncinate and the right anterior thalamic tract.

- The control group had higher radial diffusivity (RD) than GWI in anterior callosal tract and left superior longitudinal tract. The control group also showed higher mean diffusivity (MD) than cases in posterior callosal tract, bilateral superior longitudinal tracts and right anterior thalamic tracts. GWI veterans with 'in theater' mTBI history have lower MD in these tracts than GWI without mTBI group. Higher RD and MD reveals may indicate degenerative pattern.

More significant group difference patterns shown from the assessments based on the Kansas criteria than CDC

- As shown in the table 1, group assessment based on the Kansas criteria captured more multi-dimensional diffusion imaging features describing significant differences between GWI controls and cases.

Better classifier performance from high-dimensional diffusion markers

- Classifier based on Neuroimaging: From the partial dataset (12 controls and 40 GWI cases), the highest classification performance was confirmed in the multi-compartmental model indices (intra and extracellular diffusion measures, (82~88%) followed by DTI measures (~62%) outcomes for classifying GWI (fig 3). Classifier based on cortical morphometry measures revealed 50~70% accuracy in classifying GWI from the control. Clasifier based on cognitive scores revealed higher classification performance (~71%) compared to the classifiers based on DTI, morphometry and Cytokine markers.

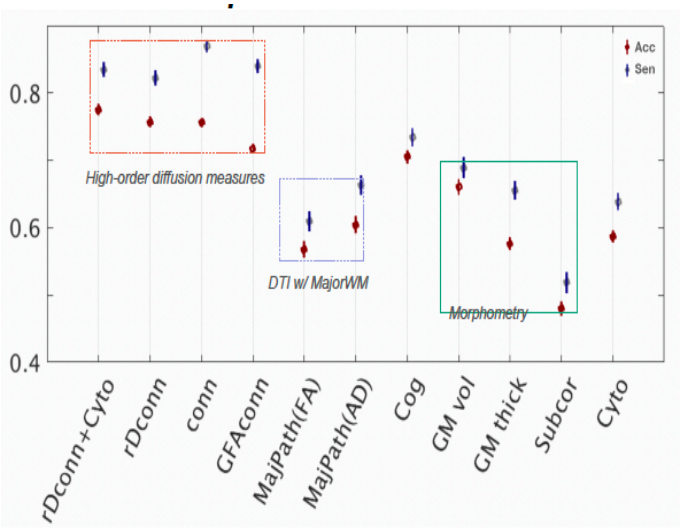

Figure 3. Benchmark testing (prelim) on the classifiers.

4) Other achievements:

By the end of the year 1, blood cytokine and the cognitive score measurements on 81 subjects were transferred from GWIC. We started building classifiers based on the blood cytokine and the cognitive score measurements from 52 subjects. This will be on-going in the 2nd project year.

We extended the multi-dimensional diffusion mapping into the GM to assess microstructural differences in the cortical and subcortical structures.

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

PI attended an international conference in June 16-22nd 2018. PI shared the preliminary findings to the neuroimaging community. PI also had extensive discussions on diffusion MRI processing with other research groups. We noticed that model based approaches on diffusion MRI processing has been strongly achieving considerable attention to the research community due to its intuitive description on the estimated parameters. PI continued the discussions with other research groups after the conference and updated DKI processing to apply the multi-compartmental diffusion modeling. Now, the image processing pipeline offers 4 different diffusion modeling (DTI, Ball-stick model, intracellular multi-compartment diffusion modeling, q-space modeling).

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

How were the results disseminated to communities of interest?

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

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

For the next reporting period, we will continue to work on building the classifiers based on different types of machine learning models combining imaging and non-imaging dataset. PI initiated technical discussions on up-to-date machine learning approaches with several research groups will also be conducted. We will also extend our discussions to find better ways to combine different types of classifiers. From this, we will bring the most cutting-edge approaches to enhance the classification outcomes of GWI case status. The best classifiers will be assessed from the planned benchmark tests. Important imaging and non-imaging features used in the classifiers will be defined throughout the project. We will also continue to work on processing the newly acquired data to produce the best quality of the datasets.

4. IMPACT:

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

Acquisition of multidimensional diffusion strength and directional encoding provided a better opportunity to apply recently developed diffusion mapping concepts along with the common DTI measures. From this novel acquisition design, we could draw extend figures on GWI as follows:

- Better understandings of recent DTI findings: DTI measures showed composite of strengthened (lower RD, lower MD, radial and mean diffusivity results in table1) or weakened (lower AD, lower FA) patterns in GWI. Although those weakened patterns could be taken as a diffusion MRI marker of WM neurodegeneration, those of opposite patterns may lead to incomplete interpretations of the results. Enhanced diffusion pattern (higher AD in GWI) was also highlighted as a potential neuroimaging marker for GWI cases in the previous reports (Chao et al., 2015, Rayhan et al., 2013). However, the authors of those studies could only make hypothetical interpretations on their findings based on their available results. We tried to tackle this problem by dissecting DTI measures into sub-compartment diffusion measures to see whether those enhanced patterns rely on enhancements on sub-compartments or not.

- Although more concrete conclusion can be made by the acquisition of the full samples, we confirmed that degenerative patterns in sub-diffusion components could be a significant source of both weakened and strengthened patterns in DTI measures of GWI. This may also support the view on the role of regional axonal atrophy resulting in artificial increases of AD mentioned in the previous study by Rayhan et al., 2013.

- Optimal parameter testing for multi-dimensional diffusion mapping: we tested different processing parameters with different combinations of diffusion encoding strength. We found that combining 3 diffusion encodings covering b-values 1000~3000s/mm^2 lowered down the modeling error. Also, optimal tract mapping based on the repeated measure of diffusion indices by varying diffusion coefficients provided higher sensitivity on detecting group specific patterns. We'll continue work on testing parameters in the project year 2 to find a standardized measurement protocol. The data itself and the protocol will be shared to the GWI research community.

- From this partial dataset, we also confirmed that GWI cases with mTBI showed stronger WM alterations than veterans without mTBI in some tracts. We also confirmed tracts that were specific to GWI case status without mTBI than those with mTBI. This may be an indication that 'in theater' exposure conditions (e.g., mTBI) is an

important neurological risk factor to describe and understand the brain health of GW veterans. This may also indicate that our proposed imaging measures have better sensitivity on describing GWI than the common DTI measures. We're extending this work to see whether the combination of risk factors (e.g., mTBI and chemical weapons exposure) relates to more severe neurodegenerative brain imaging patterns.

- This preliminary work suggests that combining machine-learning technology with multi-compartmental diffusion measures allows for better classification performances of GWI cases as well and provides a better understanding of the complex pathobiology and potential subgroupings of GWI. Some of the results were presented in the international conference (International Society of Magnetic Resonance in Medicine) on June 19, 2018.

What was the impact on other disciplines?

- Although more concrete finding can be made by the acquisition of the full samples, clear degenerative patterns in GWI captured in this work can likely be a critical information on understanding of the stress induced neurological injuries and priming of its impacts through additional risk factors.

- Further investigation of DTI indices based on the multi-compartmental diffusion model may provide increased sensitivity on describing other neurological cases.

What was the impact on technology transfer?
Since this is year 1 of the project, nothing to report yet.

What was the impact on society beyond science and technology?
Nothing to report yet.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report.

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

- We initially planned processing of 250 subject data from the Gulf war illness consortium (GWIC). However, we could only process 81 MRI scans due to the latency on subject recruitments and data collections in GWIC.

- GWIC has requested 1 year no cost extension to continuing the data collections. GWIC will use new recruitment efforts to complete recruitment goals to finalize data collections during the extended project year. We will promptly process the newly acquired data once they are transferred to the team.

Changes that had a significant impact on expenditures

- New account was finally set up on Jan 3rd 2018 from Boston university office of sponsored programs.
- PI, Dr.Koo, was appointed as an assistant professor on February 2018 from his department (Anatomy and Neurobiology). He had increased salary so his effort was adjusted from 30% to 27~28% to account this salary gap.
- PI's lab was started after the semester started and there was delay in hiring staff, research assistant at graduate student level. PI recruited undergraduate level research assistants from the computer engineering

and neuroscience department. We will continue to recruit graduate student level research assistant in the new semester.

6. PRODUCTS:

Publications, conference papers, and presentations

- Conference Presentation: Koo BB, Kimberly Sullivan, A comparison of different brain connectivity markers for classifying Gulf-war illness, International Society of Magnetic Resonance in Medicine, June 2018

Other publications, conference papers, and presentations.

- 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.
  : *We applied same diffusion analyses protocol used in the current project for testing it in the animal models. The software structure was modified to process animal model brains. We confirmed that the micro-diffusivity measures can be a sensitive marker for studying neuroinflammation.*

- Presentation on Gulf War Illness Imaging works in the External Advisory Board (EAB) meeting, June 14th 2018 (presentation slide attached).

- Koo BB, Cheng J, Little D, Steele L, Heeren T, Killiany R, Sullivan K, Preliminary Evaluation of Diffusion Imaging Features for Classifying Veterans with Gulf War Illness, International Neurological Society 2019, abstract submitted.

Other Products

- DTI measures and cortical morphometry measures from subtasks 2-1 and 2-2 described in the previous section were shared with GWIC researchers. The last update was made on June 6, 2018 and data from 62 subjects was reported at the GWIC EAB meeting on June 14, 2018. We will update additional data to GWIC once processing quality assurance process is finished.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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:	*3*
Contribution to Project:	*Ms. Cheng has performed work on data processing and organizing the imaging measurement outcomes.*
Funding Support:	*Ms. Cheng joined the team as a research intern. We recently hired Ms.Cheng as Research Assistant.*

Name:	*Khlifa Alnaim*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Mr. Alnaim has performed work on data processing and organizing the imaging measurement outcomes.*
Funding Support:	*We hired Mr.Alnaim as Research Assistant (part-time).*

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

Principal Investigator (Dr.Bang-Bon Koo):
Dr.Koo has an additional funding from NIH R01 grant as a co-investigator.
Title: Dietary quality, cognitive decline and brain health in Puerto Rican Adults
Suporting Agency: NIH-NIA (R01AG055948)
P.I.: Katherine Tucker, University of Massachusetts Lowell
Duration: 09/15/2017 – 05/31/2022
Effort: 1.2 cal. Month (Subaward: S51110000037006)

Co-investigator (Dr.Killiany):
NIH/NIAID (UC7 A2095321) has closed on Nov 2017.
Title: National Emerging Infectious Disease Laboratories Operations (PI:Corley)

NIH/NIA (R01 AG043640) has closed on Aug/2018
Title: Histopathology, Neuroimaging and Mechanism of Myeline Damage in Aging Monkey Brain (PI: Rosene)

NIH/NIA (R01 AG043478) has closed on Aug/2018
Title: Role of Curcumin on Age-related Cognitive Decline in the Rhesus Monkey (PI: Moss)

Co-investigator (Dr.Sullivan):
DoD (CDMRP/GWIRP) has closed on Aug 2018
Title: Novel Autoantibody Serum and Cerebrospinal Fluid Biomarkers in Veterans with Gulf war illness (PI: Sullivan)

DoD grant has close on June 2018
Title: D-cycloserine – A novel treatment for Gulf war illness (PI: Toomey)

Co-investigator (Dr.Heeren):
NIH/NIDDK grant has closed on Feb 2018
Title: Follow-up Glucose Testing and Timely Transition to Primary Care after Gestational Diabetes (PI: Bernstein/McCloskey)

NIH/NIAAA – R01 AA023376 has closed on June 2018
Title: The alchole policy environment and leading causes of alcohol related mortality (PI: Naimi and Xuan)

American heart association grant has closed on June 2018
Title: Behavioral cardiovascular disease prevention using informatics (PI: Quintiliani)

National institute of justice (NIJ) has closed on Dec 2017
Title: A brief intervention to prevent adolescent dating aggression perpetration (PI Rothman)

National institute of justice (NIJ) has closed on Dec 2017
Title: Evaluation of a service provision program for victims of sex trafficking (PI Rothman)

What other organizations were involved as partners?

- Organization Name: Baylor College of Medicine

Location of Organization: *One Baylor Plaza, Houston TX 77030*
Partner's contribution to the project: Consultant
Financial support: none
In-kind support *(e.g., partner makes software, computers, equipment, etc., available to project staff)*:
Discussions on the project

Facilities: None
Collaboration: responsible for managing the Texas site data and consulting on GWI symptoms on this project.
Personnel exchanges
Other.

- Organization Name: Nova Southeastern University

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

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

9. APPENDICES:

References:

Chao, L. L., Zhang, Y., & Buckley, S. (2015). Effects of low-level sarin and cyclosarin exposure on white matter integrity in Gulf War Veterans. *NeuroToxicology*, *48*, 239–248. http://doi.org/10.1016/j.neuro.2015.04.005

Rayhan, R. U., Stevens, B. W., Timbol, C. R., Adewuyi, O., Walitt, B., VanMeter, J. W., & Baraniuk, J. N. (2013). Increased brain white matter axial diffusivity associated with fatigue, pain and hyperalgesia in Gulf War illness. *PLoS ONE*, *8*(3), e58493. http://doi.org/10.1371/journal.pone.0058493

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.

1663

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

Bang-Bon Koo[1] and Kimberly Sullivan[2]

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Synopsis

Gulf War Illness (GWI) represents a cluster of multi-system chronic symptoms experienced by a third of veterans who served in the Gulf War. The exact cause of GWI remains unknown and efforts directed towards developing treatments have been hampered by the lack of meaningful objective biomarkers of the illness. Combining machine learning technology to brain connectivity imaging may allow for better understanding of the complex pathobiology of GWI. Choosing optimal imaging index should be a first step to maximize its classification performance.

Introduction

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

Methods

Training set was based on 20 GW veterans (8 GW controls and 12 GWI, based on Kansas criteria) and 18 GW veterans' data was used for the test set. We also included non-veteran aged control data (12 controls) in this study which have the same diffusion MRI data. Brain structural network was extracted from high angular resolution diffusion imaging (HARDI, spin-echo epi, 1.75/1.75/2mm voxel, 65 independent diffusion gradient directions, b-value 3000s/mm2) data collected in the Boston Gulf War Illness Consortium (GWIC). Local Brain connection was defined based on the existence white matter tracts between each of the GM regions of interests (Freesufer ROIs, total 78). Per each local brain connection, we applied the following quantifications for defining local connectivity measures: 1) total number of tracts, 2) tract mean value of microscale diffusivity[6] from generalized q-space reconstruction. We applied random decision forest classification for machine learning of the brain connectivity. Machine learning classifier was trained for different quantifications and compared with each other. Performance of the machine learning classifier was tested based on a leave one out cross validation (LOOCV) and test set classification performance.

Results

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

Discussions

Brain connectomic techniques have the potential power to uncover the underlying mechanisms of GWI. The advantage of the brain connectomics lies in their capacity to map effects of interest in both focal as well as a large-scale data points. Brain connectomics allows for processing of a large and distributed number of brain connections as well as more local and focal connections. However, our preliminary data showed that different quantification strategies in diffusion MRI can have significant impact on describing group characteristics. Lower range of diffusion encodings used in diffusion MRI (typically, around b=1000s/mm2) is the most common set up in the clinical imaging and draws mostly the fast diffusion components and it might not have enough sensitivity to detect microscopic water diffusivity changes. Better sensitivity for detecting micro-diffusivity can be obtained by adding high diffusion strength encoding (typically, b>1,800s/mm2). As shown in our preliminary observations, micro-diffusion can be a sensitive index to more diffuse changes in the brain. Since latent risk factors can possibly be present in some of the GW controls, it is understandable that classifying GWI from the non-veteran control samples could have better performances compared to the other one. However, more data is needed for concrete conclusion to be made.

Conclusion

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

Acknowledgements

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

Figures

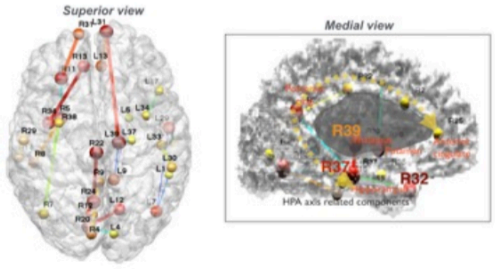

Figure 1.

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

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Short Communication

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

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ABSTRACT

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

1. Introduction

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

DoD modeling estimates 100,000 U.S. troops were potentially exposed to low level sarin and studies have found a potential impact of sarin in veterans with GWI (White et al., 2016). Accordingly, we developed a GWI mouse model incorporating exogenous corticosterone (CORT), to mimic physiological stress, and acute exposure to diisopropyl fluorophosphate (DFP), to mimic sarin nerve agent exposure experienced by GW veterans. This paradigm resulted in a marked brain-wide neuroinflammatory response in the absence of evidence of brain damage (O'Callaghan et al., 2015), highlighting the potential for these exposures to contribute to an underlying neuroinflammatory condition in GWI. In addition

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0889-1591/

to mimicking sarin, DFP shares chemical characteristics with other irreversible acetylcholinesterase, organophosphate compounds that veterans were exposed to during the Gulf War, like the insecticides chlorpyrifos and dichlorvos. As such, we have demonstrated the potential for both DFP, as a sarin surrogate, and chlorpyrifos to instigate similar neuroinflammatory responses following CORT pretreatment (Locker et al., 2017), supporting a role for these classes of compounds in the development of GWI.

To draw an in-depth relationship between altered neuroinflammatory response and sickness behavior shown in veterans with GWI, it is important to have a monitoring method which is minimally invasive and allows for the combined evaluation of immunological and neurological consequences of the toxic insults. Currently, there are different ways to examine neuroinflammatory responses *in vivo*. Measuring cytokine levels in CSF (Lenzlinger et al., 2004) may allow for the identification of proinflammatory markers in GWI. Also, positron emission tomography (PET) imaging can offer brain physiological information in GWI (Yehuda et al., 2010). However, the high invasiveness of lumbar puncture to obtain CSF and the costliness and insensitivity of PET (e.g., see Vivash and O'Brien, 2016) creates limits to the usefulness of these methods to address neuroinflammation in veterans with GWI. In order to bridge the animal to human extrapolation gap, magnetic resonance imaging (MRI) has been used to assess neural structural changes in association with an altered immune response, such as that proposed to underlie GWI. Morphometric MRI analysis of veterans with GWI confirmed overall reduction in the grey matter (GM) (Chao et al., 2010) and white matter (WM) (Heaton et al., 2007; Chao et al., 2011), as well as a reduction in hippocampal and cortical GM volumes compared to healthy veterans (Chao et al., 2010). These regional morphometric changes have been tied to alterations in brain connectivity using diffusion MRI (Rayhan et al., 2013, Chao et al., 2011). Higher axial diffusivity measures have been reported in some WM major fiber pathways in the brains of GWI-suffering veterans. These findings may indicate that there are focal spots primarily involved in illness propagation in the brain; specifically, diffusion mapping may identify underlying structural and connectivity changes between brain cells (Anwander et al., 2010; McNab et al., 2013; Leuze et al., 2012).

2. Materials and methods

In this study, we expanded our established GWI mouse model (O'Callaghan et al., 2015; Locker et al., 2017) by exposing adult male Sprague Dawley rats (Hilltop Lab Animals, Scottdale, PA, USA) to CORT in the drinking water (200 mg/L in 0.6% EtOH) for 4 days, followed by a single injection of DFP (1.5 mg/kg, i.p.). Rats were sacrificed 6 h post-DFP by either decapitation for the evaluation of cortex cytokine mRNA (n = 5 rats/group), or by a fatal dose of pentobarbital-based euthanasia solution (Fatal Plus; 300 mg/kg, i.p.) followed by transcardial perfusion with 0.9% saline and fixation with 4% paraformaldehyde (n = 5 rats/group) for MRI. Total RNA from the frontal region of one cortical hemisphere was isolated as previously described (Locker et al., 2017). Real-time PCR analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and of the proinflammatory mediators, TNFα, IL-6, CCL2, IL-1β, leukemia inhibitor factor (LIF), and oncostatin M (OSM) was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in combination with TaqMan® chemistry as previously described (Locker et al., 2017). Relative quantification of gene expression was performed using the comparative threshold ($\Delta\Delta C_T$) method. Changes in mRNA expression levels were calculated after normalization to GAPDH. The ratios obtained after normalization are expressed as fold change over corresponding saline-treated controls and two-way ANOVAs (pretreatment [water or CORT] × exposure [saline or DFP]; $p \leq 0.05$) were conducted on log transformed values using SigmaPlot v12.5 (Systat Software, Inc., San Jose, CA, USA). If statistical significance was detected between groups by two-way ANOVA, then Bonferroni post hoc analysis (statistical significance, $p \leq 0.05$) was performed to evaluate the statistical significance of all pairwise multiple comparisons. For diffusion MRI imaging analysis, paraformaldehyde-perfused brains were scanned for 10 h on a 4.7 T Bruker MRI with an applied diffusion weighted spin-echo echo planar imaging sequence (SE-EPI) with the following parameters: 500 μm isotropic voxel, coronal slice acquisition with 515 independent diffusion gradient directions using b-values up to 40,000 s/mm^2 (Wedeen et al., 2008). For each brain, five non-diffusion weighted (b0) images were averaged to perform pre-processing of the raw diffusion scans and a modified in-house processing pipeline (Koo et al., 2013) was used to perform sequential pre-processing steps on the data. Q-space imaging method (Yeh et al., 2010) were used for the reconstruction of diffusion parameters. Three dimensional probability information on the diffusion displacement was calculated in each voxel in the brain scans and then formed the spin distribution function. Micro-scale diffusivity was modeled for partial diffusion encoding length based on the weighted sum of partial spin distributions below upper bound of the diffusion displacement (Yeh et al., 2016). In this study, we applied 2 μm, 10 μm and 20 μm upper bounds for calculating the micro-scale diffusivity maps. We also calculated generalized fractional anisotropy (GFA) based on the spin distribution in q-space reconstruction. GFA has been used for quantifying microstructural integrity (similar to fractional anisotropy in diffusion tensor imaging) for q-space diffusion imaging. From the pre-processing, all diffusion indices on each brain were nonlinearly transformed to the atlas space to perform group-level statistics and all brain images were smoothed based on the Gaussian kernel (1.5 mm FWHM). Regional impacts were highlighted using unpaired group statistics between controls and the other groups (CORT, DFP, CORT+DFP) per each diffusion index separately. We conducted permutation-based random effect corrections for multiple comparison corrections with 5000 permutations to correct for possible random effects. Voxels with significant differences were defined by $p < 0.05$ and significant clusters were then mapped to the atlas to confirm anatomical information.

3. Results

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

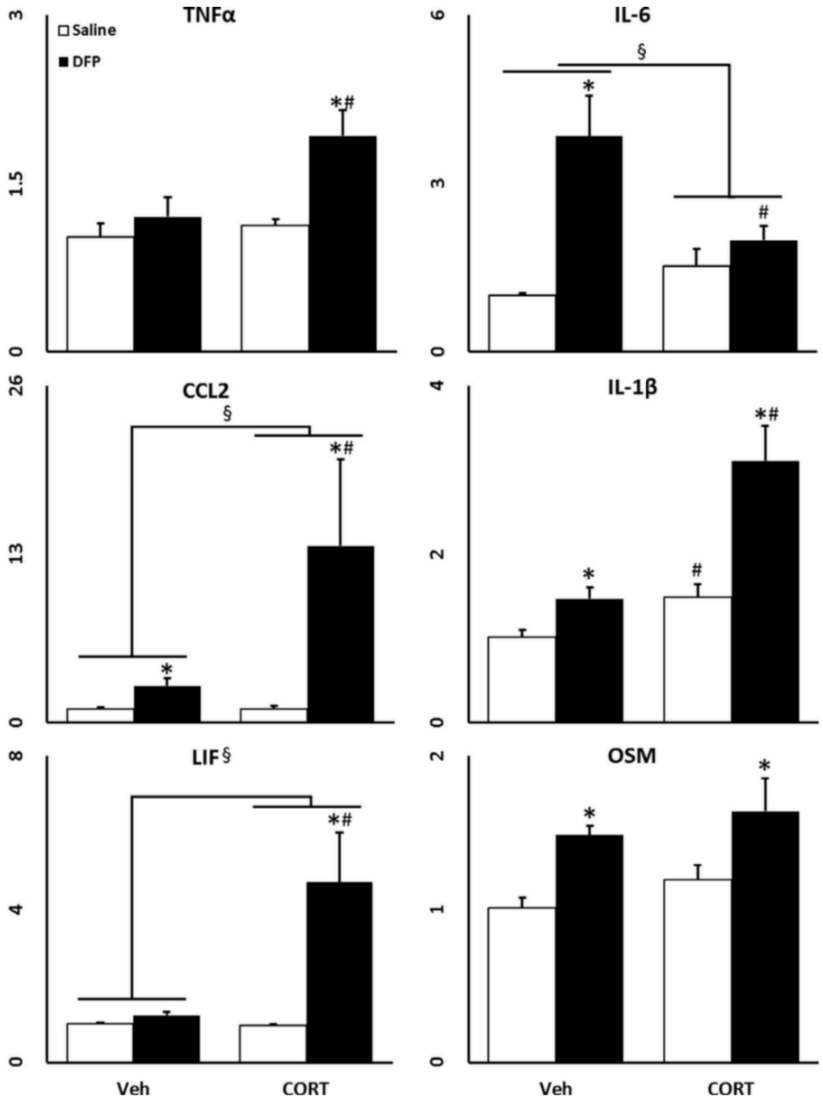

Fig. 1. Chronic CORT exacerbates DFP induced inflammation in rats. Effects of chronic CORT pretreatment (200 mg/L 0.6% EtOH in drinking water for 4 days) on DFP (1.5 mg/kg, i.p.) induced neuroinflammation in the cortex at 6 h post DFP exposure is shown with TNFα, IL-6, CCL2, IL-1β, LIF, and OSM qPCR.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

results in regions including the medial frontal and hippocampal regions (Fig. 2 third row), whereas CORT and CORT+DFP had distinct patterns compared to their micro-scale mapping results.

4. Discussion

Here, we have shown that the CORT-enhanced DFP-induced neuroinflammatory model developed for the mouse (O'Callaghan et al., 2015) can be extended to the rat and that diffusion MRI can successfully differentiate between the exposure conditions of this GWI model. We have demonstrated previously that our model of GWI is an instigation of neuroinflammation without evidence of brain damage. This has been shown previously with immunohistochemistry at relevant, short-term time points in mice (O'Callaghan et al., 2015) through an absence of positive silver stain and Fluoro-Jade markers for degenerating neurons, as well as a lack of changes in microglia or astrocytes. Similar results have been confirmed in our rat model at comparable time points (unpublished data). Therefore, at this early time point, we have shown the neuroinflammatory potential of DFP and CORT+DFP exposure in the absence of damage. Furthermore, what we have demonstrated here is that this neuroinflammatory response results in subtle, but differentiable changes in diffusion MRI, which highlights the ability to detect inflammatory-induced changes in MRI patterns early and without the requirement of severe damage to the brain tissue. Recent studies using similar diffusion parameters also reported successful discrimination of microstructural changes in neuronal or glial cell elements (Johnson et al., 2014, Blumenfeld-Katzir et al., 2011). In addition, high-order diffusion MRI successfully captured statistically significant changes in diffusion indices in a rat model of mild traumatic brain injury as early as 2 h post-injury (Zhuo et al., 2012). While the current study evaluates early

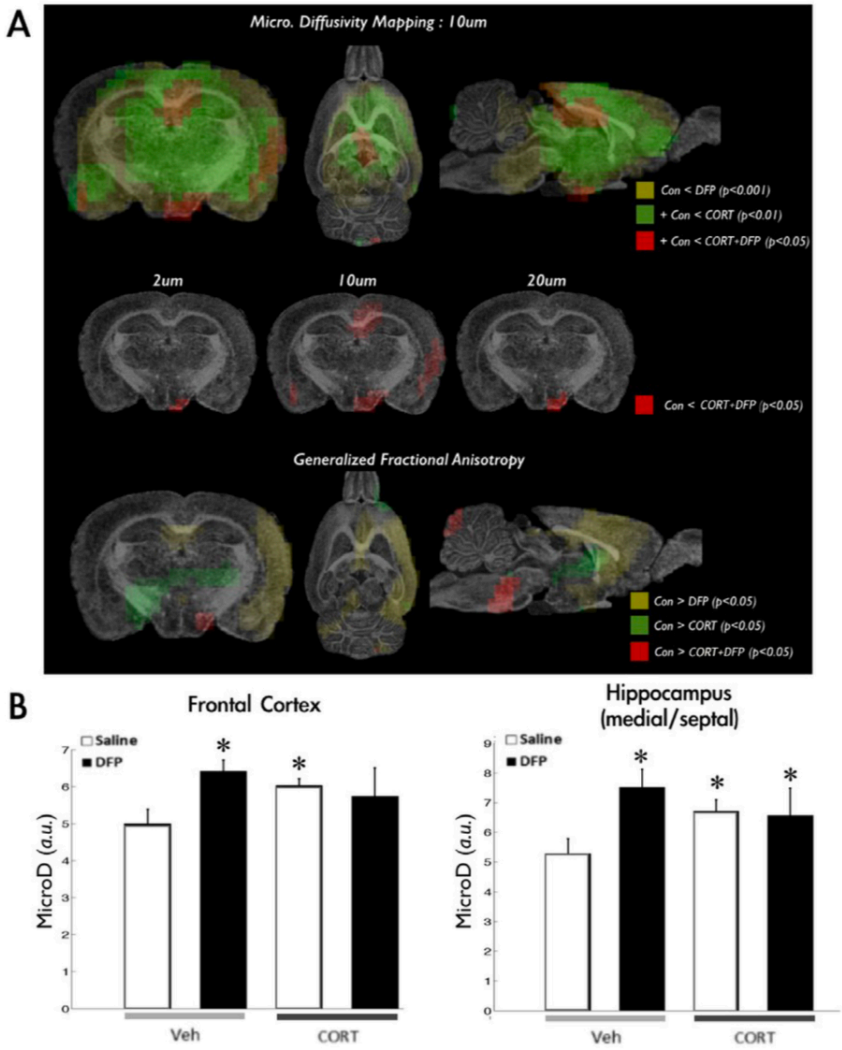

Fig. 2. Microscale diffusivity mapping in CORT + DFP treated rats. Group differences in microscale diffusivity is shown in panel A. Statistically significant differences between controls and DFP treated rats are shown in the clusters with yellow color encodings. Green clusters are overlapped to the yellow clusters and indicates differences between controls and CORT-treated rats. Red clusters are overlapped to the previous two clusters and show differences between controls and CORT + DFP treated rats. We confirmed that diffusion encoding length at 10 μm had more sensitivity to detect statistically significant group differences in between controls and CORT + DFP treated rats as shown in the second row of panel A. Generalized fractional anisotropy maps revealed distinct patterns with lower statistical thresholds compared to the micro diffusivity maps (third row in panel A). Panel B shows quantification of micro diffusivities in the frontal cortex and the hippocampus. In both graphs, white and black bar in Veh section shows average micro-diffusivity value of each region of interest in saline and DFP accordingly. Also, white and black bar in CORT section shows average micro-diffusivity value of each region of interest in CORT and CORT + DFP. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

time points in relation to a chronic illness, these conditions model what we would hypothesize to have been experienced immediately following exposure in theater or the proposed initiating events of GWI. Various studies of long-term time points that are more closely relevant to the current condition of veterans with GWI are on-going in our rodent models of GWI. Future studies will aim to address the potential for this model of "in theater" exposure conditions to progress into the chronic condition we associate with GWI and evaluate how these early diffusion changes may correlate or predict MRI results in a long-term model of GWI.

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

astrocytes. Overall, since the high-order diffusion imaging protocol is also available to 3T clinical scanners, these results help to establish high-order diffusion MRI as a means to evaluate subtle ultrastructural changes in neural cells, which may be associated with *in vivo* neuroinflammation, in veterans with GWI.

Acknowledgments

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GWIC

GWIC Imaging

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

Spring 2018 - updat

GWIC

Imaging Items to discuss

Ultimate goal to devise an Objective Diagnostic Marker of GWI

Current Strategies for developing Diagnostic marker:

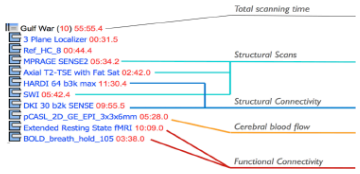
- Connectomics, Dr. Koo's multimodal imaging grant and progress to date
- Simple gray and white matter volumetric analyses
- Simple analyses of white matter pathways
- Complex analyses of white matter pathways
- Clinical impression of brain imaging outcomes – Dr. Little will discuss
- Inter-relationships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

Multimodal Imaging in Gulf-war Illness

Bang-Bon Koo, Ph.D.

Multimodal Imaging in GWI

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



+ FDG+PET scans

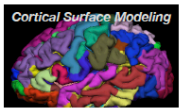
59 Cases / 13 Controls

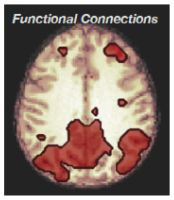
4 Cases / 3 Controls

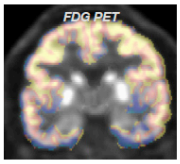
Multimodal Image Processing Pipelines

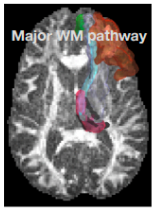
Aim I Automated Image Processing Environment

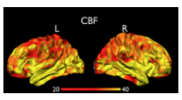
Applying current brain mapping technologies to the GWI imaging research







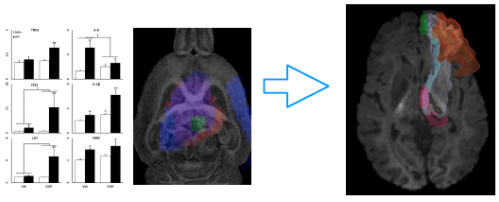




Multimodal Image Processing Pipelines

Aim II Developing Novel Image Processing Scheme

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

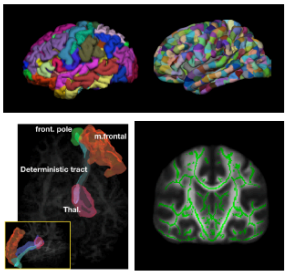


Multimodal Image Processing Pipelines

Aim II Developing Novel Image Processing Scheme

Novel Shape Deformity Assessment Algorithm on Subcortical Structures

Defining Features in Different Scales to find more sensitive markers

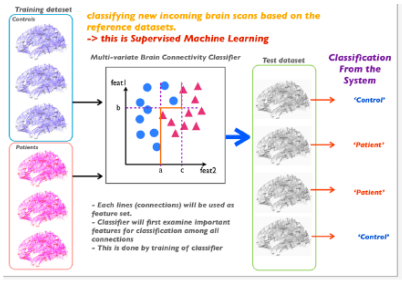


Multimodal Image Processing Pipelines

Aim III

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

Building ML classifier for Imaging, Blood immune and Cognitive Data



Multimodal Image Processing Pipelines

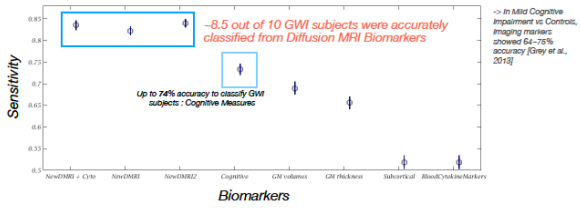
Aim III

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

Building ML classifier for Imaging, Blood immune and Cognitive Data

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

Diffusion MRI markers



Multimodal Image Processing Pipelines

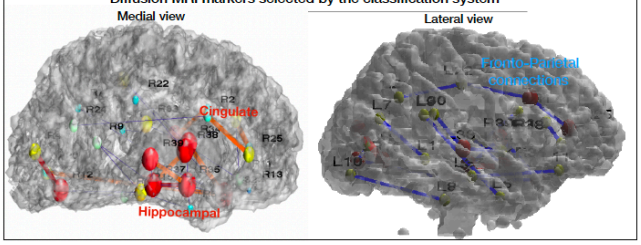
Aim III

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

Building ML classifier for Imaging, Blood immune and Cognitive Data

Cross-Validations (38 GWcase vs 12 GWcon)

Diffusion MRI markers selected by the classification system



Multimodal Imaging in GWI

Next Steps

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

BRAIN VOLUMES BY CASE STATUS N=72

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

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

INS

View Abstract

	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
	_
	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]
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	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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