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TITLE: An in Vivo Investigation of Brain Inflammation in Gulf War Illness with Integrated PET/MR Imaging

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An in Vivo Investigation of Brain Inflammation in Gulf War Illness with Integrated PET/MR Imaging

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
This project is aimed at evaluating the contribution of brain glia to the pathophysiology of Gulf War Illness, as well as fibromyalgia (a functional pain disorder characterized by similar symptoms). So far we have completed administrative and regulatory review, made substantial progress on subject enrollment, and completed some preliminary analyses. Over 400 participants have been pre-screened for the study. Of those, forty participants have completed the study, while an additional twenty-two participants were enrolled and found to be ineligible at the time of screening. Throughout the recruitment process, we have actively modified our inclusion criteria to address concerns of ambiguity as they have arisen. Recruitment was slower than anticipated initially, but has rapidly picked up over the past year and the study is now approaching completion.

15. SUBJECT TERMS
Recruitment

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1. INTRODUCTION:

In this project, we are using simultaneous magnetic resonance imaging (MR) and positron emission tomography (PET) with $^{11}$C-PBR28 – a recently developed PET ligand which binds to activated microglia with unprecedented specific-to-nonspecific binding ratio – to test the hypothesis that patients with Fibromyalgia (FM) or Gulf War Illness (GWI) demonstrate over-activation of brain microglia. Microglia are a subpopulation of macrophages known to mediate the inflammatory response of the central nervous system. While under normal conditions these cells are involved in adaptive homeostatic defense responses, such as the destruction of invading microorganisms, animal models have also provided evidence for a role of microglial activation in the development of chronic pain. Recognizing the role of these chronically active microglial cells in FM and GWI might lead to the development of new and potentially more effective treatment approaches for both conditions. Furthermore, if disease-specific patterns of microglial activation can be identified, it would improve our ability to correctly diagnose the two conditions and treat them with specificity not possible to date.

2. KEYWORDS:

Fibromyalgia; Gulf War Illness; Chronic Pain; Microglia; Neuroinflammation; Positron Emission Tomography; Magnetic Resonance Imaging

3. ACCOMPLISHMENTS:

What were the major goals of the project?

As stated in approved Statement of Work, we anticipated the following accomplishments during Year 2 of the project:

- Task 2: Subject Recruitment (months 4-24)
- Task 3: Study Visits (Behavioral and Imaging; months 5-32)
- Task 4: Data Analysis (Data Preprocessing; months 5-32)
- Task 5: Publication of Results (month 28-36)

Based on our approved quarterly enrollment targets, we anticipated enrolling and scanning 15 participants in Year 3.

What was accomplished under these goals?

During year 3, we stated that we would continue to accomplish Tasks 2-5.

Since recruitment was slower than anticipated in the beginning of this project, we began Year 3 with a focus on recruitment (Task 2). We explored alternative strategies for the recruitment of Gulf War Veterans including reaching out to veteran’s groups and utilizing social media. With the travel compensation that was added in Year 2, we were able to drastically increase the sample of both healthy and ill veterans who have been enrolled. So far, 32 veterans with Gulf War Illness and 30 healthy veterans were successfully contacted and completed the phone screening process. Of those who have been phone screened, 18 veterans with Gulf War Illness and 11 healthy veterans were identified as being potentially eligible. In this coming year, we aim to identify 8 more healthy veterans and 1 additional Gulf War Ill Veteran who can complete the study.

In year 3, Fibromyalgia recruitment and enrollment was completed for this study. Over 100 Fibromyalgia subjects were successfully contacted and completed the phone screening process. Of those subjects who were phone screened, 28 were found to be eligible to enroll in the study. Common reasons subjects were excluded from participation in the study include co-occurring major medical and psychiatric illness, as well as the use of disqualifying medications such as steroids or diazepam.
While not paid for by this award, 176 civilian healthy controls have also been prescreened as part of this study. Of those 176, 18 subjects have been enrolled in the study.

In terms of Study Visits (Task 3), so far 28 participants with fibromyalgia, 18 civilian healthy controls, 15 veterans with Gulf War illness, and 10 healthy Gulf War veterans have completed the behavioral visit process. 15 Fibromyalgia subjects, 6 civilian healthy controls, 7 healthy Gulf War Veterans, and 12 veterans with Gulf War Illness have successfully completed all study procedures. To date 22 participants were withdrawn from the study due to significant history of psychological illness (4), major medical illness (4), comorbid autoimmune disorder (2), peripheral neuropathy (2), low affinity binding genotype (6), claustrophobia (2) as well as the use exclusionary medications (3). Another 2 subjects were lost to follow up, and 1 subject withdrew from the study since she was moving out of the area. An additional 3 participants were found eligible, and are expected to complete study procedures within the next reporting period.

Initial data analysis (Task 4) has been performed comparing fibromyalgia subjects to healthy civilians and veterans with Gulf War Illness to healthy veterans.

Preliminary analyses suggest that whole brain uptake of [$^{11}$C]PBR28 is increased in subjects with fibromyalgia when compared to pain-free civilian healthy controls. Mean whole-brain SUV demonstrated a trend-level effect of group ($p = 0.053$), while there was a significant main effect of genotype ($p=0.013$) as well as a significant group X genotype ($p=0.024$) interaction. To briefly summarize, fibromyalgia subjects demonstrated increased whole brain uptake of [$^{11}$C]PBR28, and this was especially significant in those subjects who were found to be high affinity TSPO binders.

![Figure 1. PET signal group differences between FM and healthy controls](image)

A voxel-wise analysis revealed that high affinity binder fibromyalgia patients exhibited significantly elevated SUV in numerous sensory, motor, and higher-order cognitive regions compared to high affinity binding controls. There were no regions where control SUV was higher than fibromyalgia. When the mixed affinity binding subjects were included in the analysis, SUV were elevated in several of the same regions (e.g. S1, M1, cerebellum, preSMA), but at a lower statistical threshold ($p < 0.01$, uncorrected). FIQR was not significantly correlated with SUV in any region. The patterns of [$^{11}$C]PBR28 elevations in fibromyalgia support our hypothesis that microglial activation may play a role in the pathophysiology of fibromyalgia, however these findings were quite distinct from those observed in our previous chronic low back pain study. In accordance with the complex symptomatology of fibromyalgia, PET signal increases were widespread and encompassed a variety of sensory, motor and higher-order cognitive regions.
When comparing the $^{[11]}$C-PBR28 SUV data across the veterans, an elevation in the PET signal appears evident in the veterans with GWI (Figure 2). Voxelwise analyses of 7 GWI veterans compared to 18 healthy controls, show significant increases in SUVR in the cingulate cortices, S1, brainstem, superior parietal lobule, and frontal cortex.

What opportunities for training and professional development has the project provided?
The project has supported travels at several international meetings, in which the results of our study have already been presented. In keeping with the award requirements, on November 11 2017 the data will be presented at the Society for Neuroscience meeting in Washington, DC.

How were the results disseminated to communities of interest?
Poster presentations of these findings have been given at two conferences thus far: the 11th International Symposium on Functional NeuroReceptor Mapping, Boston, MA, 2016, and the 16th World Congress on Pain, the International Association for the Study of Pain (2016), Yokohama, Japan. As mentioned, the GWI findings will also be presented in November at Neuroscience 2017 in Washington, D.C.

What do you plan to do during the next reporting period to accomplish the goals?
Within the next reporting period we aim to complete all recruitment, study visits, data analyses and begin publishing our findings for this study. Given the number of subjects we were able to enroll in the last reporting period, this task is attainable in the next reporting year.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
The results of this study thus far suggest that glial activation may play a significant role in the neurobiological mechanisms underlying fibromyalgia and Gulf War Illness symptoms. If these results continue to hold true, this may point to novel therapeutic targets for treating GWI and fibromyalgia and it may also help to improve diagnostic accuracy for these debilitating disorders.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
If glial activation does play a significant role in fibromyalgia and Gulf War Illness, then this could potentially
impact drug discovery efforts for these conditions.

What was the impact on society beyond science and technology?
People with chronic pain conditions such as fibromyalgia and Gulf War Illness often experience a significant amount of stigma surrounding their diagnosis, since these conditions lack objective diagnostic tests. Many professionals still believe these disorders are more psychological than physiological in nature. It is our hope that by beginning to identify the underlying pathophysiology of fibromyalgia and Gulf War Illness, stigma surrounding these disorders will be reduced.
5. CHANGES/PROBLEMS:

All of the significant changes described below were reported to the DoD HRPO at the time of their approval by the Partners IRB. All changes have also been reported in our continuing review documents.

Changes in approach and reasons for change

1. **Modifications to inclusion/exclusion criteria**: Minor modifications to the inclusion and exclusion criteria were made in order to reduce ambiguity in the subject selection process. The need for these changes became apparent as subjects were screened and patterns in subjects’ medical histories were identified that suggested the need for the modification of certain exclusion criteria without threatening the scientific integrity of the protocol. These changes include allowing for the use of marijuana and commonly used steroid medications in subjects with Gulf War illness, excluding for sciatica in subjects with fibromyalgia and modifying the exclusion criteria for hypertension to only exclude for extreme, uncontrolled hypertension in all subjects.

2. **Modifications to study procedures**: To improve data quality, modifications were made to study procedures. Most notably, arterial blood sampling during the MR/PET scan was added back into the protocol, which also changed the study compensation. In addition, we added a mock scan to the screening visit for subjects who are uncertain of their ability to tolerate the scanning environment and we began measuring subjects’ body temperature on the day of the scan to ensure that they don’t have an elevated body temperature that could cause confounding inflammation.

3. **Modification of recruitment methods**: To increase our subject pool, Recruitment methods were modified to include: social media advertising, ResearchMatch, and the use of new Partners Healthcare databases such as patients who elected for researchers to contact them directly, and advertising on the new Partners Clinical Trials website.

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

To date, the only major delays faced were issues presented by subject recruitment. Referrals coming from the Gulf War Consortium Study were initially slower than anticipated, affecting our ability to recruit both healthy and Gulf War ill veterans. After identifying alternative recruitment methods, we were able to substantially increase enrollment in the study. In addition, by covering the cost of travel for veterans participating in the study from out of state, we were able to increase the subject pool that we are able to recruit from.

Changes that had a significant impact on expenditures

The addition of arterial line placement and blood analysis, needed to improve quantification of the PET signal, increased the cost of each scan where this data is acquired by about $750.00.

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

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations


**Website(s) or other Internet site(s)**
scholar.harvard.edu/loggia
This is the lab’s official website, hosted within the Harvard University network. The website provides an introduction to the lab’s research, recent news, and a complete list of publications.

**Technologies or techniques**
Nothing to report.

**Inventions, patent applications, and/or licenses**
Nothing to report.

**Other Products**
Nothing to report.
7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Personnel who have devoted at least 1 calendar month to the project are listed below:

<table>
<thead>
<tr>
<th>Name</th>
<th>Marco Loggia, PhD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Role</td>
<td>Principal Investigator</td>
</tr>
<tr>
<td>Researcher Identifier (ORCID ID)</td>
<td>0000-0002-8026-5265</td>
</tr>
<tr>
<td>Nearest Person Month Worked</td>
<td>3</td>
</tr>
<tr>
<td>Contribution to Project</td>
<td>Directed the project, hired and supervised personnel, collected data</td>
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<tr>
<td>Funding Support</td>
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<table>
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<tr>
<th>Name</th>
<th>Courtney Bergan</th>
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<tbody>
<tr>
<td>Project Role</td>
<td>Research Assistant</td>
</tr>
<tr>
<td>Researcher Identifier (ORCID ID)</td>
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</tr>
<tr>
<td>Nearest Person Month Worked</td>
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</tr>
<tr>
<td>Contribution to Project</td>
<td>Recruited and screened participants, collected data</td>
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<tr>
<td>Funding Support</td>
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<th>Name</th>
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<tr>
<td>Project Role</td>
<td>Co-investigator</td>
</tr>
<tr>
<td>Researcher Identifier (ORCID ID)</td>
<td>0000-0002-3249-5971</td>
</tr>
<tr>
<td>Nearest Person Month Worked</td>
<td>1</td>
</tr>
<tr>
<td>Contribution to Project</td>
<td>Provided advice on data processing</td>
</tr>
<tr>
<td>Funding Support</td>
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Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

The only investigator devoting at least 1 cal. mo. was the PI, Marco Loggia. Below are the changes in other support occurring after the beginning of the project.

New sources of support

1R61AT009306-01 (Napadow), *Optimization of brain-based mechanism supporting psychosocial aspects of acupuncture therapy – a hyperscanning fMRI study*, 0.36 cal.

R01AG053582-01 (Johnson-Akeju), *Pathophysiology of Postoperative Delirium and the Use of Biomimetic Sleep as a Treatment Strategy in the CSICU*, 0.6 cal.

5R01AR064367-04 (Edwards/Napadow), *Brain Mechanisms Underlying CBT-Related Reductions in Fibromyalgia*, 0.42 cal.
Reduced effort

1R01NS094306-01A1 (Loggia), *The role of brain glial activation in knee osteoarthritis*, from 4.56 cal. to 4.14 cal.

1R01NS095937-01A1 (Loggia), In-vivo imaging of spinal and brain glial activation in low back pain patients, from 3.24 cal to 2.76.

Terminated

Football Players Healthy Study at Harvard University, (S/C PI: Loggia), Imaging pain-related glial activation in retired professional football players

1R21NS087472 (PI: Loggia), The Role of Neuroimmune Activation in Chronic Pain and Negative Affect

NCMIC Foundation grant (PI: Loggia), Neural Correlates of Spinal Manipulative Therapy

R01 AT007550 (PI: Harris/Napadow) Neuroimaging Approaches to Deconstructing Acupuncture for Chronic Pain.

1R21NS082548-01A1 (PI: Zhang/Hooker), PET/MRI Imaging of Neuroaxial Inflammation in Sciatica Patients

What other organizations were involved as partners?

<table>
<thead>
<tr>
<th>Organization Name:</th>
<th>Brigham and Women’s Hospital</th>
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<tbody>
<tr>
<td>Location of Organization:</td>
<td>Boston, MA</td>
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<tr>
<td>Partner’s Contribution to the Project:</td>
<td>Financial Support</td>
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<td>In-kind Support</td>
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<td>Dr. Lee has been working with project staff on the project.</td>
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<th>Organization Name:</th>
<th>Boston University School of Public Health – Gulf War Illness Consortium</th>
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<td>Dr. Sullivan has been working with project staff on the project</td>
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8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Nothing to report

QUAD CHARTS:
Nothing to report
9. APPENDICES:

A. Does glial cell activation have a role in fibromyalgia? A $[^{11}C]PBR28$ PET study

**INTRODUCTION**

Fibromyalgia (FM) is characterized by widespread pain, fatigue, and other symptoms. FM pathophysiology is poorly understood, but central mechanisms have been implicated. While some have postulated a role for neuroinflammation / glial activation in FM, no study has ever demonstrated its occurrence in FM.

We have recently shown that patients with chronic low back pain demonstrated increased [11C]PBR28 SUV in regions of increased TSPO expression. In accordance with the complex symptomatology of FM, PET signal increases were widespread and encompassed a variety of sensory, motor and higher-order cognitive regions. In our previous chronic low back pain study, we were able to observe significant increases even in non-normalized SUVs, indicating that the group differences in TSPO expression may be substantial enough to overcome the large within-group variance in [11C]PBR28 data.

**METHODS**

- 13 FM patients and 14 pain-free healthy controls received a $[^{11}C]PBR28$ PET/MR brain scan.
- All subjects were genotyped for the Ala147Thr TSPO polymorphism, which predicts $[^{11}C]PBR28$ binding affinity (i.e., Ala/Thr=High affinity binders, HAB; Ala/Ala=Low affinity binders, LAB).
- Integrated PET/MRI scans conducted on a 3T Siemens PET/MRI scanner with $[^{11}C]PBR28$ for 60 minutes.

**RESULTS**

FM patients display higher mean whole-brain $[^{11}C]PBR28$ SUVs

- Mean whole-brain SUV demonstrated a trend-level effect of group ($p = 0.053$), a significant main effect of genotype ($p = 0.013$), and a significant group x genotype interaction ($p = 0.024$).

- Post-hoc tests revealed that the group effect was driven by HAB subjects, as group differences in whole-brain SUV were significant only in HABs ($p = 0.04$).

Voxelwise comparison identifies widespread regions of increased $[^{11}C]PBR28$ signal in FM

- In sum, these data provide preliminary evidence of increased global TSPO expression in FM, suggesting the occurrence of widespread glial activation. If validated in a larger dataset, these observations would support the exploration of glial-based therapeutic approaches for FM.

**DISCUSSION AND CONCLUSIONS**

- Fibromyalgia patients exhibited higher $[^{11}C]PBR28$ SUV across the entire brain, compared to controls.
- The patterns of $[^{11}C]PBR28$ elevations in FM were quite distinct from those observed in our previous chronic low back pain study. In accordance with the complex symptomatology of FM, PET signal increases were widespread and encompassed a variety of sensory, motor and higher-order cognitive regions.
- TSPO PET imaging, the large interindividual variability in the global signal makes the utilization of intensity normalizing strategies (e.g., whole brain normalization) desirable, in order to identify effects that may be obscured by large within-group variance. The fact that we were able to observe significant increases even in non-normalized SUVs, indicates that the group differences in TSPO expression may be substantial enough to overcome the large within-group variance in $[^{11}C]PBR28$ data.
- Nonetheless, future investigations will need to validate alternative normalization methods, such as the use of a suitable pseudo-reference region, in order to improve the characterization of the $[^{11}C]PBR28$ signal in FM. The presence of significant differences in whole-brain SUV across groups indicates that the use of whole brain uptake to intensity-normalize $[^{11}C]PBR28$ data, previously adopted for the study of different pathologies, may not be appropriate for FM.
- In sum, these data provide preliminary evidence of increased global TSPO expression in FM, suggesting the occurrence of widespread glial activation. If validated in a larger dataset, these observations would support the exploration of glial-based therapeutic approaches for FM.