AWARD NUMBER: W81XWH-17-1-0488

TITLE: Glutamate Receptor and Kynurenine Pathway Functioning in the Pathobiology of Gulf War Illness

PRINCIPAL INVESTIGATOR: Marijn Lijffijt

RECIPIENT: Baylor College of Medicine
One Baylor Plaza
Houston, TX 77030-3411

REPORT DATE: OCTOBER 2020

TYPE OF REPORT: Annual Technical Progress Report

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

REPORT DOCUMENTATION PAGE		Form Approved OMB No. 0704-0188
1. REPORT DATE OCTOBER 2020	**2. REPORT TYPE** Annual report	**3. DATES COVERED** 30 SEP 2019 – 29 SEP 2020
4. TITLE AND SUBTITLE Glutamate Receptor and Kynurenine Pathway Functioning in the Pathobiology of Gulf War Illness		**5a. CONTRACT NUMBER** W81XWH-17-1-0488 **5b. GRANT NUMBER** GW160077 **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S) Marijn Lijffijt, Ph.D.E-Mail: marijn.lijffijt@bcm.edu E-Mail: marijn.lijffijt@bcm.edu		**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Baylor College of Medicine One Baylor Plaza Houston, TX 77030-3411		**8. PERFORMING ORGANIZATION REPORT**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012		**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

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

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project has 2 aims: (i) examine the involvement in veterans with Gulf War Illness of a neural excitatory state as a consequence of impaired brain immune, neuron and glia functioning using biomarkers obtained from cerebrospinal fluid (CSF) in 1990-1991 Gulf War veterans with (n=46) and without (n=23) GWI, and (ii) examine involvement in veterans with GWI of a neural excitatory state defined as increased glutamatergic receptor functioning by testing the effect of a single infusion of 0.5 mg/kg of N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine on gamma band EEG (for NMDAR target engagement), other EEG markers, and on symptoms of Gulf War Illness in 19 cases. Outcomes will provide evidence of an expected neural excitatory and pro-inflammatory state in cases that could predispose to neuronal damage via NMDAR hyperactivation through kynurenine pathway activation, and will provide evidence in humans of possible effects of temporarily blocking NMDAR's with a subanesthetic dose (0.5 mg/kg) of ketamine.

15. SUBJECT TERMS
Inflammation; kynurenine pathway; quinolinic acid; microglia; astrocytes; symptoms; Gulf War Illness; ketamine; cerebrospinal fluid; subject recruitment; No cost extension

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	10	

Standard Form 298 (Rev. 8-98)

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

This project has 2 aims: (i) examine the involvement in veterans with Gulf War Illness of a neural excitatory state as a consequence of impaired brain immune, neuron and glia functioning using biomarkers obtained from cerebrospinal fluid (CSF) in 1990-1991 Gulf War veterans with (n=46) and without (n=23) GWI, and (ii) examine involvement in veterans with GWI of a neural excitatory state defined as increased glutamatergic receptor functioning by testing the effect of a single infusion of 0.5 mg/kg of N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine on gamma band EEG (for NMDAR target engagement), other EEG markers, and on symptoms of Gulf War Illness in 19 cases. Outcomes will provide evidence of an expected neural excitatory and pro-inflammatory state in cases that could predispose to neuronal damage via NMDAR hyperactivation through kynurenine pathway activation, and will provide evidence in humans of possible effects of temporarily blocking NMDAR's with a subanesthetic dose (0.5 mg/kg) of ketamine.

2. **KEYWORDS:** .

Inflammation; kynurenine pathway; quinolinic acid; microglia; astrocytes; symptoms; Gulf War Illness; ketamine; cerebrospinal fluid; subject recruitment; No cost extension

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

What were the major goals of the project?

Aim 1 is to determine biomarkers of central inflammation in cerebrospinal fluid (CSF), and associate those biomarkers with GWI symptoms.

The goal of sub-task 1 is to obtain approval of the human subject protocol by the Baylor College of Medicine (BCM) IRB, Michael E. DeBakey VA Medical Center (MEDVAMC) R&D, and DoD HRPO that had to be reached at the end of month 6 (the end of March 2018).

The goal of sub-task 2 is to start recruitment efforts in month 7 (April 2018) which continues to the end of month 28.

The goal of subtask 3 is to start research procedures in eligible veterans, which was projected start in month 7 and continue to end of month 28. At the end of September 2019 (end of month 30), the projected number of subjects enrolled in the study for aim 1 was 69.

Aim 2 is to evaluate involvement of NMDAR functioning in GWI.

The goal of sub-task 1 is to obtain approval of the human subject protocol by the Baylor College of Medicine (BCM) IRB, Michael E. DeBakey VA Medical Center (MEDVAMC) R&D, and DoD HRPO that had to be reached at the end of month 30 (the end of September 2019).

The goal of sub-task 2 is to start recruitment efforts in month 31 (October 2019) which continues to the end of month 41.

The goal of subtask 3 is to start research procedures in eligible veterans, which was projected start in month 30 and continue to end of month 41. At the end of December 2019 (end of month 33), the projected number of subjects enrolled in the study for aim 2 was 10.

What was accomplished under these goals?

AIM 1: determine biomarkers of central inflammation in cerebrospinal fluid (CSF), and associate those biomarkers with GWI symptoms.
Sub-task 1, and subtask 2 objective 1 were completed in 2018.

Sub-task 2

Objectives 2 and 3 in 2019: Veteran recruitment and identification, and research procedures. Key outcomes: Similar to the last reporting period, recruitment for the CSF study was very slow. We were able to recruit 2 more veterans for research procedures. The current grant would have ended September 30 2020, but we requested and received a No-Cost Extension (NCE) that bridges this grant to September 30 2021. We received IRB and HRPO approval to continue recruitment for AIM 1 in the NCE period. However, starting March 2020, the Michael E. DeBakey VA Medical Center halted all in-person human subject research because of COVID-19. In that period we have not enrolled any more subjects. AIM 1 called for the recruitment of 69 veterans (46 cases and 23 controls); we collected CSF of 7 subjects. To mitigate the sample, we entered in this reporting period into Material Transfer Agreements (MTA's) with Drs. Sullivan and Baraniuk to obtain CSF samples collected as part of their GWI studies. Dr. Sullivan will provide us with 4 CSF samples, whereas Dr. Baraniuk will provide us with 44 CSF samples. This means that at the end of the current reporting period (2020) we have access to 55 CSF samples, 29 cases and 26 controls. The samples will be send to Dr. Lena Brundin for assaying in January 2021. She is expected to report the findings to us in February 2021.

AIM 2: Evaluate involvement of NMDAR functioning in GWI.
Sub-task 1.

Objective 1: Obtain approval of the human subject protocol by the Baylor College of Medicine (BCM) IRB, Michael E. DeBakey VA Medical Center (MEDVAMC) R&D, and DoD HRPO. Key outcomes: We obtained all approvals in this reporting period but not before the local COVID-related halt on all in-person human subject research.

Sub-task 2:

Objective 1: starting March 2020, the Michael E. DeBakey VA Medical Center halted all in-person human subject research because of COVID-19; that facility started staged reopening at the beginning of October 2020. We will start recruiting for the AIM 2 study at the beginning of January 2021.

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

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

We plan on completing both of our goals during the next reporting period.

For goal 1, we completed this reporting period two Material Transfer Agreements to obtain CSF samples from GW veteran cases and controls from the BBRAIN biorepository. Our collaborator and co-I Dr. Brundin will analyze the samples and report to us the findings by February 2021. Submission of the manuscript with the findings is planned for April 2021.

For goal 2, we received this reporting period IRB and HRPO approval for single dose administration of ketamine to test effects of NMDA receptor reactivity and on GWI symptoms. The Michael E. DeBakey VA Medical Center is in the final stage of lifting the halt on in-person human subject research instigated in March 2020 because of COVID-19. We are currently in the process of contacting veterans who participated in our CSF study to ask if they are interested in participating in the ketamine challenge

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

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

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

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

Changes in approach and reasons for change

By contrast to our original proposal that called for 69 samples of CSF (46 cases and 23 controls) for AIM 1, we have now access to 55 samples with an about even distribution between cases and controls. We expect that these samples are sufficient for our goals to examine select brain biomarkers of inflammation and neural excitation.

By contrast to our original proposal for AIM 2 that called for 19 cases and 19 controls to receive an intravenous infusion of 0.5 mg/kg ketamine in a double-blind study, we amended the proposal to now include 21 cases who will participate in an open-label ketamine study. We will assess reactivity of ketamine on neural excitation measured with EEG, and explore effects of ketamine on GWI symptoms up to one week after ketamine infusion. We have received IRB and HRPO approval for these changes. The reason for these changes is because of the COVID-19 halt of human subject research.

Finally, because is the problem with recruitment of veterans for the invasive CSF study, our study for AIM 1 was significantly delayed, which also delayed the start of the project for AIM 2. We applied for, and received, a No Cost Extension of this grant so that we can continue this grant until September 30, 2021.

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

We are confident that AIM 1 will be completed by the beginning of February 2021, after which we will publish the outcomes that we expect to report upon in the Final Progress Report.

For AIM 2, the major problem is the halt on all in-person human subject research at the Michael E. DeBakey VA Medical Center (MEDVAMC). The MEDVAMC started staged opening at the beginning of October 2020, but because of illness at our department and the time that we require to set up study equipment (including order medication by the MEDVAMC research pharmacy), we expect to start recruitment in January 2021. We expect the first veterans to get ketamine in the middle of January 2021, and push to enroll at least 2 veterans for this study each week until completing enrollment. The data from the study, including EEG, will be checked for data quality during and immediately after data collection. We expect to present the data of AIM 2 in the Final Progress Report and expect to submit a manuscript for publication in October or November 2021.

Changes that had a significant impact on expenditures

We do not foresee a significant impact on expenditures

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

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?

No change

Name:	Marijn Lijffijt, PhD
Project Role:	Research Coordinator
Nearest Person Month Worked:	6
Contribution to Project:	Dr. Lijffijt has contact with the IRB and HRPO, and submits all required documentations to those organizations for (continued) approval. Dr. Lijffijt has also been able to obtain Material Transfer Agreements with two other GWI researchers to obtain their CSF samples from the BBRAIN biorepository which complement our own samples. Finally, Dr. Lijffijt participated in subject recruitment and in research activities.
Name:	Bylinda Vo-Le, MS.
Project Role:	Research Coordinator
Nearest Person Month Worked:	6
Contribution to Project:	Ms. Vo-Le has recruited veterans, screened veterans, and administered many of the questionnaires and other tasks that are part of this project

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

Nothing to report

What other organizations were involved as partners?

Organization name:	Michael E. DeBakey VA Medical Center
Location of organization:	Houston, TX
Partner's contribution to the project:	Facilities: study staff uses the partner's facilities for subject recruitment and project activities. Collaboration: we collaborate with partner's staff who also have GWI projects for bimonthly meetings to discuss subject recruitment and study progress.
Organization name:	Michael E. DeBakey VA Medical Center
Location of organization:	Houston, TX
Partner's contribution to the project:	Facilities: study staff uses the partner's facilities for subject recruitment and research project activities.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ebrap.org** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

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