AWARD NUMBER: W81XWH-17-1-0488

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

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CONTRACTING ORGANIZATION: Baylor College of Medicine, Houston, TX

REPORT DATE: October 2021

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE		Form Approved OMB No. 0704-0188
1. REPORT DATE October 2021	**2. REPORT TYPE** Annual	**3. DATES COVERED** 30Sep2020-29Sep2021
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** USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	Unclassified	10	**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified			

Standard Form 298 (Rev. 8- 98)

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1. INTRODUCTION:

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:

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.

The goal of sub-task 2 is to start recruitment efforts which continues to the end of the study.

The goal of subtask 3 is to start research procedures in eligible veterans.

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.

The goal of sub-task 2 is to start recruitment efforts until the end of the study.

The goal of subtask 3 is to start research procedures in eligible veterans.

What was accomplished under these goals?

AIM 1: determine biomarkers of central inflammation in cerebrospinal fluid *CSF), and associate those biomarkers with GWI symptoms.

In the last period, which is now in the No Cost Extension period of the grant, a total of 73 samples were assayed by Dr. Brundin at the Van Andel Institute. Analyses used samples from 59 veterans with GWI (mean age = 48.63; 9 women) and 12 healthy veteran and non-veteran controls (mean age = 47.92; 2 women). Measured were CSF tumor necrosis factor alpha (TNF-alpha), interferon gamma (IFN-gamma), interleukin 1beta (IL-1beta), IL-2), IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, eotaxin-1, GFAP, YKL-40, tryptophan (TRP), kynurenine (KYN), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), quinolinic acid (QUIN), picolinic acid (PIC), nicotinamide (NTA), nicotinic acid (NIC), anthranilic acid (AA). IFN-gamma, IL-1beta, IL-12p70 and IL-13 could not be included in the analyses because most individuals had undetectable concentrations. The distributions were non-normal and could not be normalized with transformations. This prevented the use of Bayesian statistical approaches.

Proinflammatory TNF-alpha and IL-6, and anti-inflammatory IL-10 did not differ between groups. No differences between groups were found in kynurenine pathway metabolites, including pro-inflammatory and microglia-specific QUIN or anti-inflammatory astrocyte-specific KYNA. Finally, glia-associated chemokine IL-8 and astrocyte-specific glial fibrillary acidic protein (GFAP) did not differ between groups. These outcomes are at odds with some of the human studies that suggested dysregulation of pro- and anti-inflammatory pathways using biomarkers obtained from blood plasma or serum, and from animal studies.

Non-parametric statistics (Welch's t-test for two independent samples) revealed an increase in pro-inflammatory interleukin-4 (IL-4) ($t = 2.76$, uncorrected $p = .01$) and increases in astrocyte-specific biomarkers eotaxin-1 ($t = 3.32$, $p = .002$) and YKL-40 ($t = 3.17$, $p = .004$) in veterans with GWI compared to healthy controls. The increase in eotaxin-1 agree with an increase in eotaxin-1 blood plasma or serum in veterans with GWI reported by another study. Enhanced YKL-40, the increase in IL-4 and eotaxin-1 suggests an upregulation of a pro-inflammatory pathway perhaps through astrocyte activation.

A weakness of the current study is the limited number of controls (n=12). The 12 veterans were recruited, however, across four individual studies across three different study sites and indicates the difficulty in obtained controls for an invasive procedure. We are proposing a novel study to purchase control CSF samples from U.S. biobanks to complement the 12 samples that we have. Finally, we were approved by the Michael E. DeBakey VA Medical Center (Houston) for funding to send left-over samples of 66 veterans to a lab for determination of neurofilament light, a biomarker of neuronal damage, to explore inflammatory pathways more fully in veterans with GWI. BBRAIN, the GWI biorepository, has approved our request to use the samples for that analysis.

AIM 2: Evaluate involvement of NMDAR functioning in GWI.

We received approval of the protocol by the local IRB and VA R&D, and from HRPO. Due to COVID-related restrictions at the MEDVAMC to have in-person contact with subjects, the first GWI patient successfully received an intravenous infusion of ketamine on 07/21/2021. We continue recruitment for that study once the second No Cost Extension has been approved. Because of miscommunications with the Baylor College of Medicine, Sponsored Programs send our NCE request on 01/04/2022 that we had send them in September 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?

For aim 1, samples were collected at three sites for four different GWI-related projects. We were able through material Transfer Agreements and with the help of BBRAIN to obtain 73 samples for AIM-1 analyses. We had originally planned to write a manuscript of the outcomes and have that submitted at the end of 2021. However, because we found out that we only have 12 controls, we are at risk for type-1 and type-2 errors. In fact, the number of controls could go down further considering that some subjects (who were not originally collected by us) had a very low age. I am now working with co-I Dr. Lea Steele on a dataset that will contain subjects with a pre-specified minimum age. In addition, in 2022 we will have the samples analyzed for neurofilament light. After the receipt of neurofilament light we plan on writing a report on the findings, as well as to use our outcomes to obtain funding from the DoD or the VA to purchase from USA commercial biorepositories 55 CSF samples of controls matched to the case group on gender, age and race/ethnicity.

For aim 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. We are waiting for approval of the NCE and, for the MEDVAMC, for approval of the renewal of the project. We plan to continue recruitment and testing after approvals.

4. IMPACT:

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:

Changes in approach and reasons for change

Nothing to Report

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

AIM 1: We found out that we only have 12 controls. This number could go down further considering that some subjects (who were not originally collected by us) had a very low age. I am now working with co-I Dr. Lea Steele on a dataset that will contain subjects with a pre-specified minimum age. After the receipt of neurofilament light data we plan on writing a report on the findings, as well as to use our outcomes as preliminary data to obtain funding from the DoD or the VA to purchase from USA commercial biorepositories 55 CSF samples of controls matched to the case group on gender, age and race/ethnicity.

AIM 2: The MEDVAMC has been allowing in-person meetings with research subjects again. The current problem is a delay in approval of the NCE which is needed to free up the funds for salaries and payment for research procedures, and well as a delay in project approval by the MEDVAMC R&D likely because of the transition to a new system to submit renewal requests of projects.

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:	7
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:	4
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:

QUAD CHARTS:

9. APPENDICES: