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TITLE: Glutamate receptor and Kynurenine pathway functioning in the pathobiology of Gulf War Illness

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14. ABSTRACT

This project examines inflammatory pathway biomarkers obtained from cerebrospinal fluid (CSF) collected from 46 1990-1991 Gulf War veterans with and 23 without Gulf War Illness, and test effects of N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine on symptoms of Gulf War Illness in 19 cases and 19 controls. In the last period we focused on subject recruitment. On November 29, 2018, accessed information of local veterans from the 1990-1991 Gulf War registry. Using registry information, we send recruitment letters to 92 veterans as of December 31, 2018. Of the 92, we contacted by phone 42 veterans; an additional 5 veterans were referred to the study through other sources. Of the 47 veterans, 12 were phone screened. Two met initial inclusion criteria and agreed to a lumbar puncture for CSF collection. One was a screen fail due to excluding medical conditions, and one is completing screening. Our project is behind schedule of subject recruitment, in part because veterans do not agree to the lumbar puncture. In the coming year we will continue subject recruitment, as well as amend the protocol to access other resources for study-related biomarkers, including (i) biomarkers collected from blood, and (ii) accessing CSF biorepositories.

15. SUBJECT TERMS Inflammation; kynurenine pathway; quinolinic acid; microglia; astrocytes; symptoms; Gulf War Illness; ketamine; cerebrospinal fluid; subject recruitment; protocol amendment					
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1. INTRODUCTION:

This project has 2 aims: (i) to examine the inflammatory state and possible consequences on neuronal and glia functioning using biomarkers obtained from cerebrospinal fluid in 1990-1991 Gulf War veterans with (n=46) and without (n=23) Gulf War Illness, and (ii) to test the effect of a single infusion of 0.5 mg/kg of N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine on gamma band EEG, a measure of NMDAR target engagement) and symptoms of Gulf War Illness in 19 cases and 19 controls. Outcomes will provide evidence of an expected pro-inflammatory state in cases that could predispose to neuronal damage via NMDAR hyperactivation, and possible effects of temporarily blocking NMDAR hyperactivation with a subanesthetic dose of ketamine.

2. KEYWORDS:

Inflammation; kynurenine pathway; quinolinic acid; microglia; astrocytes; symptoms; Gulf War Illness; ketamine; cerebrospinal fluid; subject recruitment; protocol amendment

3. ACCOMPLISHMENTS:

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

What were the major goals of the project?

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

Aim 1 is to determine biomarkers of central inflammation in cerebrospinal fluid (CSF), and associate those biomarkers with GWI symptoms. Aim 1 is divided in six sub-tasks, three of which fall in the last period.

The goal of sub-task 1 was 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 was to start recruitment efforts in month 7 (April 2018) which continues to the end of month 28.

The goal of subtask 3 was 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 December 2018 (end of month 15), the projected number of subjects enrolled in the study was 25.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

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

Sub-task 1

Objective 1: Approval of the protocol by the BCM IRB (Month 3, December 2017). Key outcome: Initial IRB approval September 13, 2017. IRB approval of HRPO-recommended amendments on January 10, 2018 (Month 4).

Objective 2: Approval of the protocol by the MEDVAMC R&D (Month 6, March 2018). Key outcome: Approval by the MEDVAMC R&D on October 16, 2017 (Month 1).

Objective 3: Approval of the protocol by the HRPO (Month 6, March 2018). Key outcome: Approval of the protocol by HRPO on 02/28/2018.

This completed all milestones for Sub-task 1

Sub-task 2

Objective 1: Request access to the Gulf War Registry (this was NOT part of the SOW). Key outcome: I submitted the request for access to the Gulf War Registry on March 14, 2018. The required Agreement for Release of VHA data was signed on June 01, 2018. The request was approved on June 22, 2018. MEDVAMC IT was unable to give me access to my account and email until October 24 (!). After downloading the registry on October 26 (month 13), we noticed that only names were provided, not the requested addresses and phone numbers. I resubmitted the request and got access to all requested variables on November 29, 2018 (month 14).

Objective 2: Veteran recruitment and identification (Month 7, April 2018, to Month 28). Key outcomes: Recruitment was delayed until month 13 (October 2018). We started sending recruitment letters to veterans identified in the October 26 Gulf War Registry using further information in MEDVAMC medical records. More letters were send using identifiers from the November 2018 registry. We have send recruitment letters to 92 veterans as of December 31, 2018 (month 15). Of the 92, we were able to contact 42 veterans by phone; an additional 5 veterans were referred to the study through other sources. Of the 47 veterans, 12 showed interest in the study and were phone screened. Two met initial inclusion criteria and agreed to a lumbar puncture for CSF collection. Of those veterans, one was a screen fail due to excluding medical conditions, and one is in the process of completing screening. Our project is behind schedule of subject recruitment. We were supposed to have enrolled 25 subjects by the end of month 15 assuming start of enrollment in month 7. Instead we started real recruitment efforts in month 14. Enrollment is further complicated that the majority of contacted veterans are unwilling to have a lumbar puncture performed. We will address possible solutions in the next sections of this Annual Report.

Sub-task 3

Objective 1: Hiring and training a research coordinator for the study (this was not part of the initial SOW) (month 4, January 2018). Key outcome: Bylinda Vo-Le, MS, was hired in March 2018 (month 6) as coordinator.

Objective 2: Training (this was not part of the initial SOW) (month 4). Key outcome: Bylinda and I were trained at the end of month 6 (April 2018) on the use of study-specific measures.

Objective 3: Order study-associated consumables (month 8, May 2018). Key outcome: The lumbar puncture kits have been delivered (month 9). Other consumables (study material; binders; envelopes) are ready for use.

Objective 4: Procedures associated with subject study procedures, including the informed consent process, screening procedures and study procedures. Key outcome: The Standard Operating Procedure (SOP) is in place.

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

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

Nothing to report.

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

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Recruitment is problematic in large part because prospective subjects are not consenting to the lumbar puncture. I will use a two-pronged approach to accomplish obtaining useful study-related outcomes, which are inflammatory markers in CSF (Aim 1).

1) I am in the process of submitting an amendment to HRPO (followed by the BCM IRB and MEDVAMC R&D) to obtain blood instead of CSF, with CSF being the preferred medium that subjects can consent to and blood as an alternative method if subjects are unwilling to give CSF. Although potentially less reliable than CSF inflammatory biomarkers, similar biomarkers of brain inflammation in blood are being used throughout psychiatric and neurologic studies.

2) Together with co-I Dr. Lea Steele I am researching the possibility to obtain CSF samples from existing sources such as biobanks and laboratories currently collecting CSF from veterans with and without Gulf War Illness.

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

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

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

Nothing to report.

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

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

Nothing to report.

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

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

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

Nothing to report.

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

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

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

Nothing to report.

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

As discussed earlier, prospective subjects are not consenting to the lumbar puncture, the primary medium to examine biomarkers of brain inflammatory pathways. To address this concern I will:

1) submit an amendment to HRPO (followed by the BCM IRB and MEDVAMC R&D) to obtain blood instead of CSF, with CSF being the preferred medium that subjects can consent to and blood as an alternative method if subjects are unwilling to give CSF. Although potentially less reliable than CSF inflammatory biomarkers, similar biomarkers of brain inflammation in blood are being used throughout psychiatric and neurologic studies.

2) research, together with co-I Dr. Lea Steele, the possibility to obtain CSF samples from existing sources such as biobanks and laboratories currently collecting CSF from veterans with and without Gulf War Illness.

Both options have not yet been submitted for approval. Option 1 will be submitted for approval before the end of month 16 (January 2019). Until approval of the amendment we stop further recruitment of veterans who are in the 1990-1991 Gulf War Registry.

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.

Please see comment above

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

Nothing to report.

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

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

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

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

Nothing to report.

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

Nothing to report.

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

Nothing to report.

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

Nothing to report.

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

Nothing to report.

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

Nothing to report.

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

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

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

Example:	
Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Bylinda Vo-Le, MS
Project role:	Research Assistant
Nearest person month worked:	6 (0.5 FTE; this will increase depending on project demands)
Contribution to project:	Ms. Vo-Le has used the registry to send letters to prospective subjects, has pre-screened and screened subjects after obtaining informed consent, performed all study procedures to obtain subject eligibility, collaborated closely with our colleagues to obtain subjects for our project, participated in outreach efforts in the main lobby of the Michael E. DeBakey VA Medical Center (the local VA where she and I hold a WOC position).

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Nothing to report.

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

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

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

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.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: ***N/A***

QUAD CHARTS: N/A

9. APPENDICES: *N/A*