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TITLE: Therapeutic Intervention of Glial-Mediated Enhancement of Neuroinflammation in an Established Model of GWI

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CONTRACTING ORGANIZATION: Centers for Disease Control and Prevention
Morgantown, WV 26505

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Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Centers for Disease Control
and Prevention
1095 Willowdale Road
Morgantown, WV 26505

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The main objectives of this project is to expand upon the current understanding of the effects of high physiological stress on nerve agent exposure to identify how the duration of corticosterone (CORT) exposure impacts the long-term endpoints of our GWI model, which glial cell types are involved in the neuroinflammatory phenotype associated with GWI, and whether cell-specific or targeted neuroinflammatory treatment can alleviate GWI symptomatology. We aim to 1) evaluate the duration of CORT exposures necessary to produce the neuroinflammatory phenotype seen in an established, clinically relevant, GWI mouse model; 2) identify the contribution of astrocytes and/or microglia in the development of enhanced neuroinflammation in our GWI model; and 3) test the efficacy of potential therapeutic interventions that target glia and reduce neuroinflammation in our GWI model.

15. SUBJECT TERMS

Gulf War Illness; Diisopropyl Fluorophosphate; Corticosterone; Lipopolysaccarides; Neuroinflammation; Neurodegeneration; Gliosis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Unclassified	Unclassified	Unclassified	Unclassified	17	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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

The main objectives of this project is to expand upon the current understanding of the effects of high physiological stress on nerve agent exposure to identify how the duration of corticosterone (CORT) exposure impacts the long-term endpoints of our GWI model, which glial cell types are involved in the neuroinflammatory phenotype associated with GWI, and whether cell-specific or targeted neuroinflammatory treatment can alleviate GWI symptomatology. We aim to 1) evaluate the duration of CORT exposures necessary to produce the neuroinflammatory phenotype seen in an established, clinically relevant, GWI mouse model; 2) identify the contribution of astrocytes and/or microglia in the development of enhanced neuroinflammation in our GWI model; and 3) test the efficacy of potential therapeutic interventions that target glia and reduce neuroinflammation in our GWI model.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Gulf War Illness; Diisopropyl Fluorophosphate; Corticosterone; Lipopolysaccarides; Neuroinflammation; Neurodegeneration; Gliosis

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?
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.

Research-Specific Tasks:	**Anticipated Months to complete**	**% Completion**
Major Task 1: Obtain Protocol Approval:		
Subtask 1: Obtain NIOSH ACUC approval for animal use in the proposed project.	1-3	100%
Subtask 2: Once NIOSH ACUC approval is obtained, ACURO approval of the animal protocols will be completed.	3-6	100%
Milestone(s) Achieved: Approved Protocols	6	100%

Specific Aim 1: Expand the characterization of the established GWI model in mice		
Major Task 2: Model GWI in adult male C57BL6/J mice		
Subtask 1: Initiate GWI phenotype by exposing mice to chronic CORT treatment for either 4 or 7 day increments with DFP on day 5 or 8 respectively. Continued exposures to CORT for either 4 or 7 day increments, respectively, every other week for 30 or 90 days. [2 cohorts of mice with 5 mice/group and 1 cohort of mice with 3 mice/group X 10 groups at 2 time points (30 and 90 days)] = 260 mice]	7-12	75%
Subtask 2: Challenge mice in GWI model with LPS at 30 or 90 days. Mice will be sacrificed 6 hours after LPS exposure.	9-12	75%
Subtask 3: Sacrifice by microwave irradiation (to measure organophosphorylation) [100 mice], decapitation (to measure neuroinflammation) [100 mice], or transcardial formalin perfusion (to obtain neurohistological evidence of glial phenotype) [60 mice] and sample analysis.	12-15	75%
Milestone(s) Achieved: Determine organophosphorylation and neuroinflammatory consequences of the GWI phenotype under challenge conditions at 30 and 90 days. Determine if 30 day time point is sufficient to pilot GWI phenotype.	15-18	75%
Milesone(s) Achieved: Prepare manuscript for publication	17-20	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.

Major Task 1: Obtain protocol approval.

Both IACUC and ACURO approval of animal protocols was obtained.

Major Task 2: Model GWI in adult male C57BL6/J mice.

Subtasks 1, 2, and 3 have been begun in this first year of funding. Initial experiments for neuroinflammation and neurohistological analyses in male C57BL6/J mice have been completed. Mice have been ordered and kits obtained to complete the organophosphorylation experiments.

Results:

Neuroinflammation: Mice were treated with CORT (200 mg/L 0.6% EtOH) in the drinking water for 7 days. On the 8th day, DFP (4 mg/kg, i.p.) was administered and animals were given CORT in the drinking water for 7 days in week 3 and 5 at the end of which mice were given LPS (0.5 mg/kg, s.c.) and sacrificed by decapitation 6 hours later and prepared for qPCR analysis of inflammatory cytokines and chemokines.

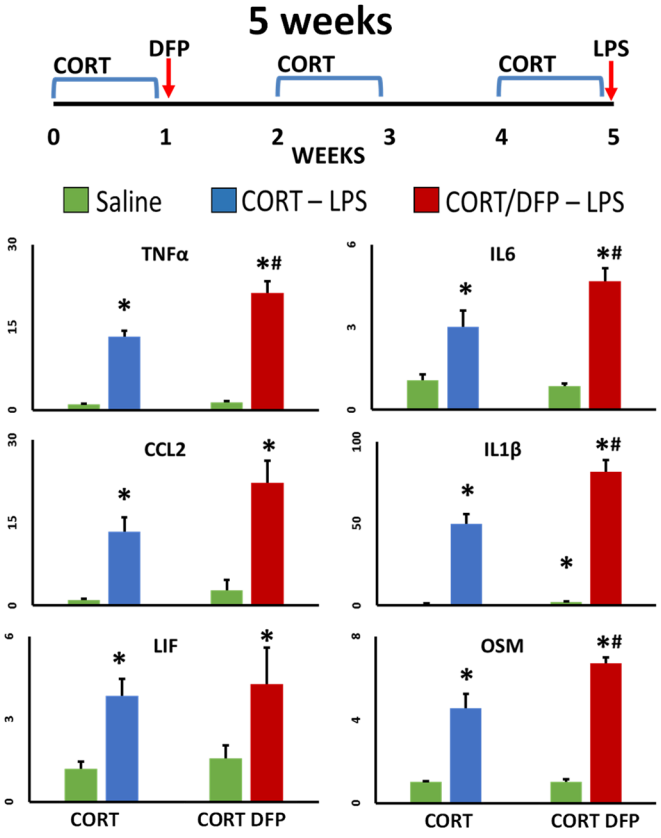

Figure 1: The combination of CORT and DFP provide an initiating event such that subsequent systemic inflammatory challenge is exacerbated.

We have found that prior chronic exposure to the stress hormone CORT can enhance the neuroinflammation caused by sarin surrogate DFP (O'Callaghan et al., 2015, Locker et al., 2017) providing evidence for combined exposures to CORT and DFP as an animal model for the initiating event producing GWI pathobiology. Here, we have expanded upon this initiating event to show that the combination of CORT and DFP produce a sensitivity to subsequent systemic inflammatory challenge (Figure 1). These data provide evidence for an animal model of Gulf War Illness that mimics the dormant phenotype that emerges when faced with an injury, infection, or illness.

Neurohistology: Mice were treated with CORT (200 mg/L 0.6% EtOH) in the drinking water for 7 days. On the 8th day, DFP (4 mg/kg, i.p.) was administered and animals were given CORT in the drinking water for 7 days in week 3 and 5 at the end of which mice were given LPS (0.5 mg/kg, s.c.) and sacrificed by transcardial formalin perfusion 24 hours later. Brains were removed and sent to FD Neurotechnologies for microglia, astrocyte, and neurodegeneration staining.

Previously we have shown acute exposure to the initiating event producing GWI pathobiology (CORT and DFP) did not produce significant glial changes in morphology or neurodegeneration (O'Callaghan et al., 2015). Here, we have used that same initiating event with a systemic inflammatory challenge at 5 weeks. While we have slides in hand for these experiments, only a subset of the microscopic images have been taken and thus an incomplete dataset is shown in the figures below.

The astrocyte response to the 5 week GWI phenotype is shown in Figure 2. The combination of CORT and DFP was able to create a pathology in which a subsequent, systemic low dose LPS challenge was able to produce astrocyte hypertrophy at 24 hours after exposure. The panels on the right depict representative control and challenged GWI phenotype treated astrocytes at high magnification to highlight the morphological difference in the astrocytes under these conditions.

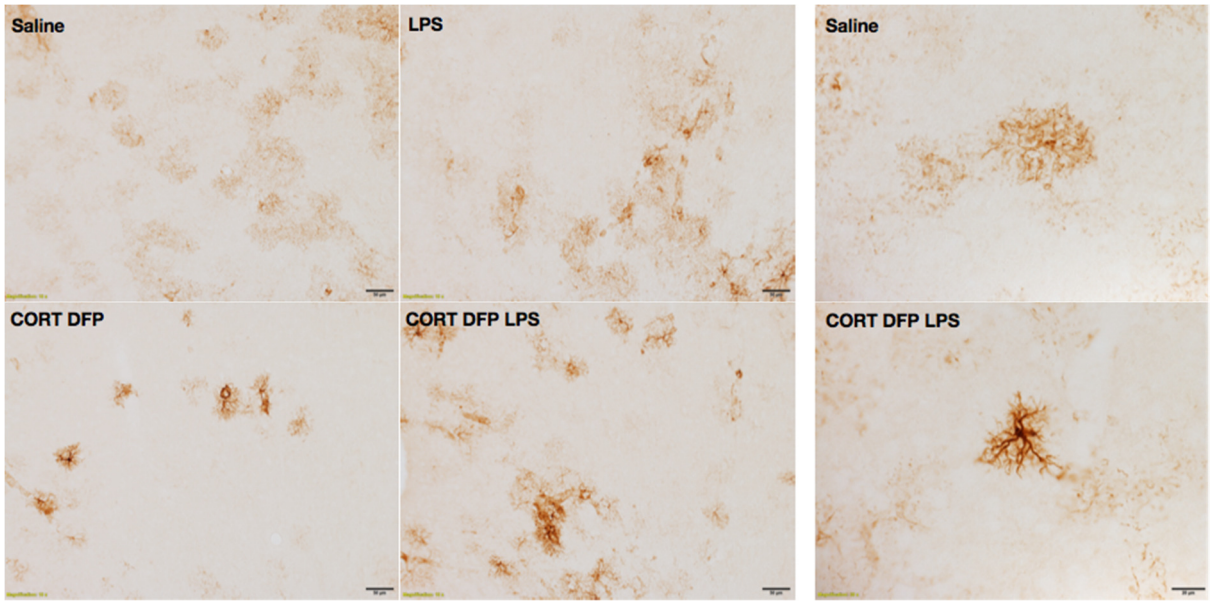

Figure 2: Astrocytes in the front Cortex appear to have increased hypertrophy (shown at 20x magnification)

Representative astrocytes (saline and CORT DFP LPS) are shown at 60x magnification in the panels to the right.

Both Silver and Fluro-Jade B neurodegeneration stains were used in this paradigm. Neither Silver (Figure 3) nor Fluoro-Jade B (Figure 4) stains showed significant changes in our model.

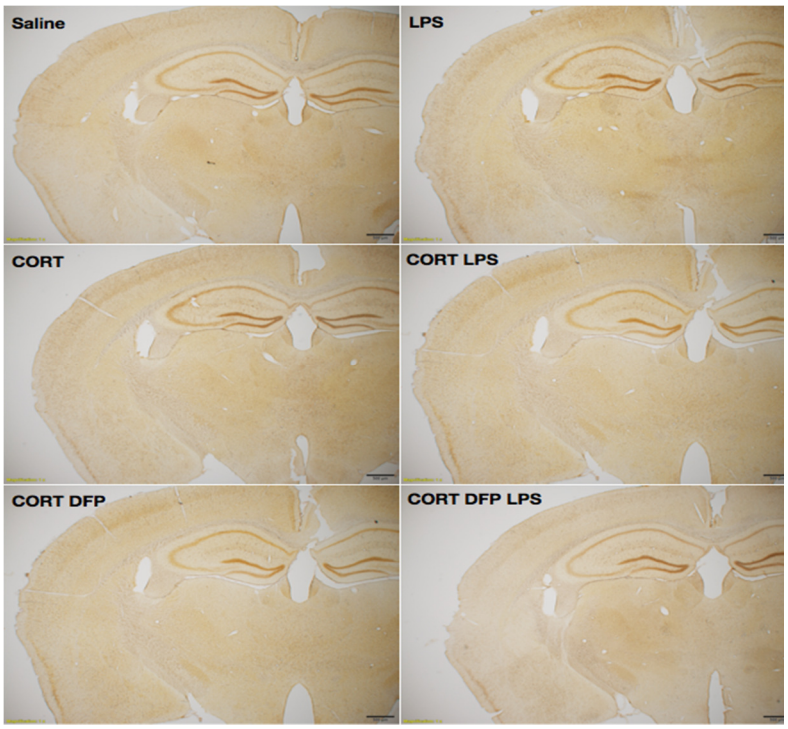

Figure 3: Silver stain for neurodegeneration 2x hippocampus.

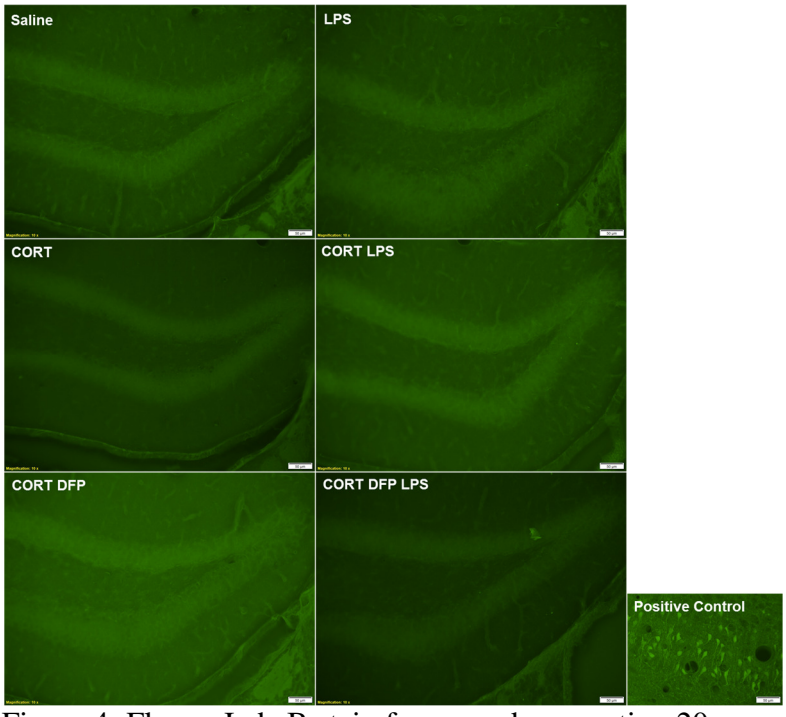

Figure 4: Fluoro-Jade B stain for neurodegeneration 20x magnification, hippocampus dentate gyrus shown. Positive Control image included at right.

Animals have yet to be completed for long term analysis of histology or organophosphorylation endpoints. Recent findings from collaborations in another GWI project have concluded that the short term time point has significant overlap with human blood samples of patients with GWI. Therefore, based on this information and the neuroinflammatory end point data shown above we have concluded that this short term animal model can be used to model the GWI phenotype (this is the milestone for task 1 in the statement of work). As such, the second Specific Aim: "Identify the contribution of astrocytes and/or microglia in the development of enhanced neuroinflammation in our GWI model," has been started by increasing animal numbers in our ALDH1L1 bacTRAP transgenic colony and pilots utilizing the microglial wipeout diet (PLX3397) that are currently underway.

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.

Attendance at the Society for Neuroscience annual meeting, Winter Conference on Brain Research, and Society of Toxicology annual meeting provided training and professional development in neuroscience and toxicology.

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

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

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."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

1) Phosphorylation states will be measured in acute CORT+DFP and GWI phenotype (CORT+DFP with subsequent systemic inflammatory challenge) paradigms to discover

phosphorylation sites other than acetylcholinesterase that may be contributing to GWI pathology.

2) The number of ALDH1L1 bacTRAP mice in our breeding colony is being increased to meet the demand for these animals in year two of this project. Next generation sequencing will be performed utilizing samples from these animals to identify the contribution of astrocytes in GWI pathology.

3) The microglial wipeout drug has been obtained and formulated into rodent food. This diet will be fed to the animals and a pilot experiment will be run to ensure its efficacy. It will then be used in the ALDH1L1 bacTRAP mice to identify, indirectly, the contribution of microglia in GWI pathology.

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?
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 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
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Initially this grant included data obtained using a 30 day paradigm. It has been found that a 5 week exposure paradigm has much better results and allows for a week of CORT administration in which both DFP and LPS exposures are not directly interacting with the CORT treatment. This has developed as an excellent period to test treatment compounds, because the treatment is given at a time long enough after DFP to avoid the question of whether the initiation of the GWI phenotype has been prevented and long enough before LPS challenge to avoid the question that the treatment of interest directly impacted the mechanism of action of LPS inflammation itself. This is ideal, because: 1) we cannot treat veterans with GWI to prevent the condition that they currently have; and 2) our goal is to discover a treatment that reverses the underlying pathobiology of GWI, not treat symptoms or "flare-ups."

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.

IACUC approval took longer than expected. This situation has been resolved. Pilot experiments will be given sufficient lead to time to prevent delays in completing the tasks in the statement of work in the future.

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.

All protocols have been approved by the IACUC and ACURO.

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **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.*

Kelly KA, Michalovicz LT, Miller JV, Broderick G, Craddock TJ, Miller DB, O'Callaghan JP: Resetting homeostasis in Gulf War Illness, a computational biology hypothesis tested in a translational mouse model. Abstract & Poster. Society of Toxicology, Baltimore, MD March 2017

Kelly KA, Locker AR, Michalovicz LT, Vrana JA, Vashishtha S, Broderick G, Miller DB, O'Callaghan JP: A mouse model of Gulf War Illness reveals a primed neuroinflammatory response to subsequent systemic inflammatory challenge. Abstract & Poster. Society for Neuroscience, San Diego, CA November 2016

Michalovicz LT, Kelly KA, Locker AR, Vrana JV, Miller DB, O'Callaghan JP: Using novel ALDH1L1 bacTRAP technology to evaluate the astrocyte-specific response to Gulf War Illness-related exposures. Abstract & Poster. Society for Neuroscience, San Diego, CA November 2016.

Broderick G, Vashishtha S, Russell L, Michalovicz L, Kelly KA, Vrana JA, Locker AR, Barnes ZM, Craddock TJA, Fletcher MA, Klimas NG, Miller DB, O'Callaghan JP, Morris M: Stress potentiation of the brain's immune response to neurotoxic exposure in the field: an animal model of Gulf War Illness. Abstract & Poster. International Association for Chronic Fatigue Syndrome/Myalgeic Encephalomyelitis Research and Clinical Conference: Emerging Science and Clinical Care, Fort Lauderdale, Florida October 2016.

- **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. In addition to a description of the technologies or techniques, describe how they will be 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. State whether an application is provisional or non-provisional and indicate the application number. 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;*
- *biospecimen 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:	**Kimberly Kelly**
Project Role:	PI
Research Identifier (e.g. ORCID ID):	0000-0002-1146-3137
Nearest person month worked:	12
Contribution to Project:	Designed and implemented experiments, analyzed and interpreted data, preparation of abstracts/manuscripts
Funding Support:	CDC-NIOSH

Name:	James O'Callaghan
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID):	0000-0001-8497-4598
Nearest person month worked:	3
Contribution to Project:	Designed experiments, interpreted data, preparation of abstracts/manuscripts
Funding Support:	CDC-NIOSH

Name:	Lindsay Michalovicz
Project Role:	post-doctoral fellow
Research Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	3
Contribution to Project:	implementation of experiments, analyzed data, preparation of abstracts/manuscripts
Funding Support:	CDC-NIOSH

Name:	Brenda Billig
Project Role:	Lab manager
Research Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	implementation of experiments
Funding Support:	CDC-NIOSH

Name:	Christopher Felton
Project Role:	laboratory technician
Research Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	1
Contribution to Project:	implementation of experiments
Funding Support:	CDC-NIOSH

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.*

Nothing to report

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://ers.amedd.army.mil** 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.