AWARD NUMBER: **W81XWH-16-1-0717**

TITLE: Identification of Causes and Treatments for Chronic Pain in a Model of Gulf War Illness

PRINCIPAL INVESTIGATOR: **Peter Grace**

CONTRACTING ORGANIZATION: **University of Texas M D Anderson Cancer Center**
Houston, TX 77030

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TYPE OF REPORT: **Annual**

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

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6. AUTHOR(S)

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5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

University of Texas M D Anderson Cancer Center
Houston, TX 77030

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14. ABSTRACT
Over the past 3 decades, 25-30% of veterans from the 1990-1991 Gulf War have consistently reported numerous unexplained chronic health symptoms affecting their quality of life, which has been termed Gulf War Illness (GWI). Musculoskeletal pain is a principal symptom of GWI, and affects up to 17% of veterans with GWI. Pain is not only a major quality of life burden for the patient, but exacts a substantial economic toll in terms of direct health care costs and absenteeism. Epidemiological studies have identified exposure to acetylcholinesterase inhibitors as a potential cause of GWI, as well as the stress of war as a sensitizing condition that might predispose soldiers to GWI. Rodent models have recapitulated the symptoms of GWI with exposure to these agents. With only one exception, pain has not been assessed in animal studies, despite being a principal symptom of GWI. Several mechanisms underlying GWI symptoms have been posited from animal studies, including neuroinflammatory signaling. However, no neuroimmune pharmacotherapies have been assessed in any model of GWI.

15. SUBJECT TERMS-

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU		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.

   Musculoskeletal pain is a principal symptom of Gulf War Illness (GWI), and affects up to 17% of Gulf War veterans. This project uses a rat model to investigate the neurobiological basis of pain in GWI and to test putative neuroimmune pharmacotherapies. Aim 1 determines whether a model of GWI sensitizes rats to a subsequent subthreshold nociceptive stimulus, resulting in induction of sustained pain-relevant behaviors. Aim 2 assesses neuroinflammatory markers in the pain neuraxis during the initiation and maintenance phase of musculoskeletal pain. Aim 3 uses pharmacological inhibitors against neuroinflammatory pathways to determine whether they reverse musculoskeletal pain in GWI.

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

   Musculoskeletal pain, Gulf War Illness, neuroinflammation, TLR4, microglia, S1PR1, p38

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.

   Major Task 1: (Preliminary activities) Obtain approval from the University of Texas MD Anderson Cancer Center Institutional Animal Care & Use Committee (IACUC) **and ACURO** for all animal work in the proposal
   Milestone 1: *animal protocol is approved by MD Anderson IACUC and ACURO.*

   Major Task 2: Acidic/normal saline administered 14 days after final DFP injection for behavior.
   Corticosterone (CORT)/vehicle administration followed by diisopropylfluorophosphate (DFP)/vehicle; intramuscular acidic vs. normal saline delivered. Behavioral testing (von Frey, Hargreaves, conflict avoidance) until all rats return to baseline thresholds.

   Major Task 3: Acidic/normal saline administered 180 days after final DFP injection for behavior.
   CORT/vehicle administration followed by DFP/vehicle; intramuscular acidic vs. normal saline delivered. Behavioral testing (von Frey, Hargreaves, conflict avoidance) until all rats return to baseline thresholds (months 2-11).

   Milestone 2*: the behavioral effect of GWI on sensitization to musculoskeletal pain is defined.*

Major Task 4: Acidic/normal saline administered 14 days after final DFP injection for tissue analyses.
CORT/vehicle administration followed by DFP/vehicle; intramuscular acidic vs. normal saline delivered. Euthanasia and necropsy at days 5 and 20 post acidic/normal saline, and tissue analysis performed (ELISA, Western Blot, LC-MS).

Major Task 5: Acidic/normal saline administered 180 days after final DFP injection for tissue analyses.
CORT/vehicle administration followed by DFP/vehicle; intramuscular acidic vs. normal saline delivered. Euthanasia and necropsy at days 5 and 20 post acidic/normal saline, and tissue analysis performed (ELISA, Western Blot, LC-MS).

Milestone 3*: the association between GWI-induced musculoskeletal pain and neuroinflammation, as well as the optimal timepoint for immuno-modulator administration in Aim 3 is defined.*

Major Task 6: Acidic/normal saline administered 14 days after final DFP injection; pharmacological reversal.
CORT followed by DFP; intramuscular acidic saline delivered. Seven-day administration of FTY720, (+)-Naltrexone, losmapimod, or minocycline. Behavioral testing (von Frey, Hargreaves, conflict avoidance) every 3-7 days until all rats return to baseline thresholds. Serum samples analysed by ELISA.

Major Task 7: Acidic/normal saline administered 180 days after final DFP injection; pharmacological reversal.
CORT followed by DFP; intramuscular acidic saline delivered. Seven-day administration of FTY720, (+)-Naltrexone, losmapimod, or minocycline. Behavioral testing (von Frey, Hargreaves, conflict avoidance) every 3-7 days until all rats return to baseline thresholds. Serum samples analysed by ELISA.

Milestone 4*: Pharmacological attenuation of GWI-induced musculoskeletal pain is defined*

Milestone 5*: Final report is completed; publication of 1-2 peer reviewed papers*

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.

<u>Major Task 2</u>: Acidic/normal saline administered <u>14</u> days after final DFP injection for behavior: COMPLETED

The aim of this task is to determine whether a model of GWI (CORT+DFP) sensitizes rats to a subsequent subthreshold nociceptive stimulus (a single intramuscular (IM) acidic saline administration), resulting in induction of sustained nociceptive behaviors. As shown in **Figure 1**, we report that acidic saline dramatically induces ipsilateral hindpaw allodynia in the GWI model (CORT/DFP). This allodynia recovers 40 days after acidic saline injection. Neither CORT/DFP or acidic saline alone substantially reduce withdrawal thresholds. The potentiation of hindpaw allodynia by acidic saline was limb-specific; the withdrawal threshold of the contralateral paw was not influenced by the IM acidic saline injection.

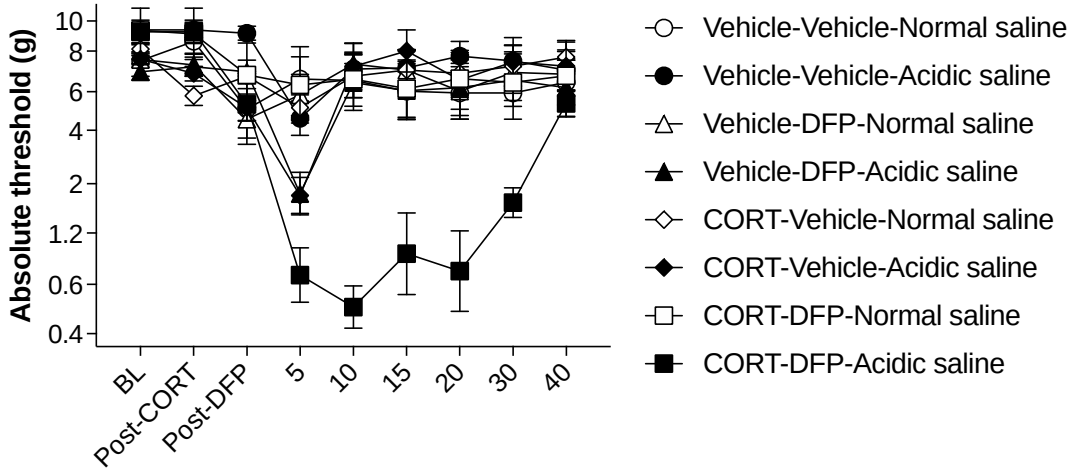

Figure 1. Baseline nociceptive measures were performed, and rats received either CORT or vehicle control in the drinking water for 7 days. On day 7, rats received a single subcutaneous injection of DFP or vehicle. Rats were allowed to recover from the acute DFP toxicity for 1 week and, after pre-injection behavioral testing, received a single, unilateral IM injection of acidic saline (pH 4) or normal saline (pH 7) into the gastrocnemius muscle. Von Frey testing of the ipsilateral paw followed on days 5, 10, 15, 20, 30 and 40 after acidic/normal saline injection.

Thermal hyperalgesia, as measured by the Hargreaves test, did not develop after any combination of treatments (**Figure 2**).

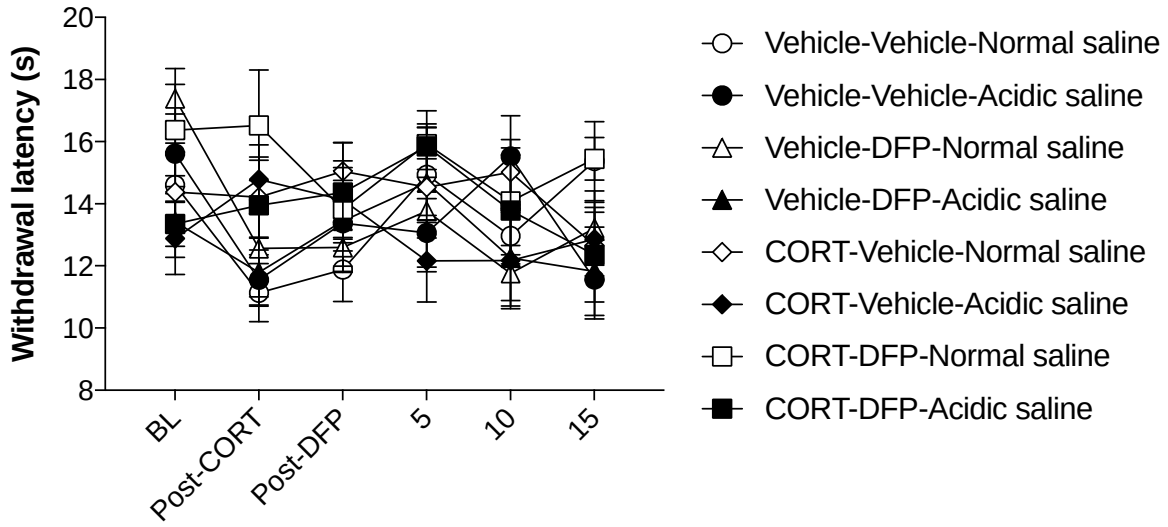

Figure 2. Baseline nociceptive measures were performed, and rats received either CORT or vehicle control in the drinking water for 7 days. On day 7, rats received a single subcutaneous injection of DFP or vehicle. Rats were allowed to recover from the acute DFP toxicity for 1 week and, after pre-injection behavioral testing, received a single, unilateral IM injection of acidic saline (pH 4) or normal saline (pH 7) into the gastrocnemius muscle. Hargreaves testing of the ipsilateral paw followed on days 5, 10, and 15 after acidic/normal saline injection.

In **Figure 3**, we report results from the mechanical conflict-avoidance task. Without using funds from this grant, we optimized the assay in three different neuropathic pain models, as there is not yet an accepted method. The protocol was performed over two days, with each day consisting of three trials. The procedure per trial was as follows: 1) rats were placed inside the light compartment with the lid closed, the light off, and the exit door closed, 2) after 20 seconds the light was turned on, 3) after 15 seconds the exit door was opened if/when the rat faced the exit, 4) the rat was able to freely explore all 3 compartments for 5 minutes, 5) rats were returned to their home cage. On day 1 (day 13 after acidic saline injection), the probe height was set to 0 mm for all three trials. On day 2 (day 13 after acidic saline injection), the probe height was set to 0 mm for the first trial, and then raised to 4 mm for the second and third trials. Corticosterone treatment appeared to have the greatest impact on latency to cross to the dark chamber; acidic saline treatment did not increase the latency to cross.

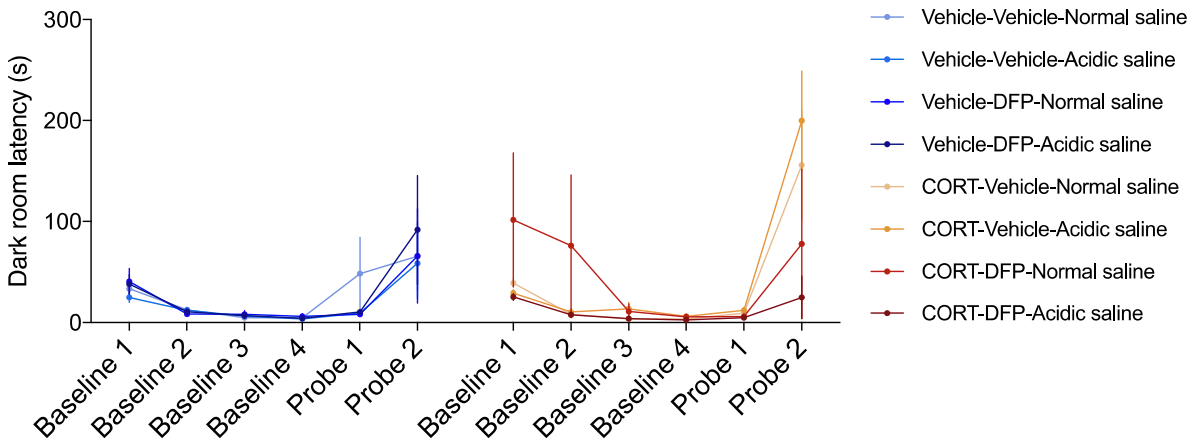

Figure 3. Rats received either CORT or vehicle control in the drinking water for 7 days. On day 7, rats received a single subcutaneous injection of DFP or vehicle. Rats were allowed to recover from the acute DFP toxicity for 1 week and then received a single, unilateral IM injection of acidic saline (pH 4) or normal saline (pH 7) into the gastrocnemius muscle. Mechanical conflict avoidance testing occurred on days 13 and 14 after acidic/normal saline injection.

<u>Major Task 3</u>: Acidic/normal saline administered <u>180</u> days after final DFP injection for behavior. IN PROGRESS

The aim of this task is to determine whether a model of GWI (CORT+DFP), with monthly re-exposure to corticosterone, sensitizes rats to a subthreshold nociceptive stimulus (a single intramuscular (IM) acidic saline administration) when given 6 months after DFP, resulting in induction of sustained nociceptive behaviors. This task is still in progress, and will be completed in the next month.

In **Figure 4**, we report that acidic saline modestly induces ipsilateral hindpaw allodynia in the GWI model (CORT/DFP). Neither CORT/DFP or acidic saline alone substantially reduce withdrawal thresholds. The potentiation of hindpaw allodynia by acidic saline was limb-specific; the withdrawal threshold of the contralateral paw was not influenced by the IM acidic saline injection.

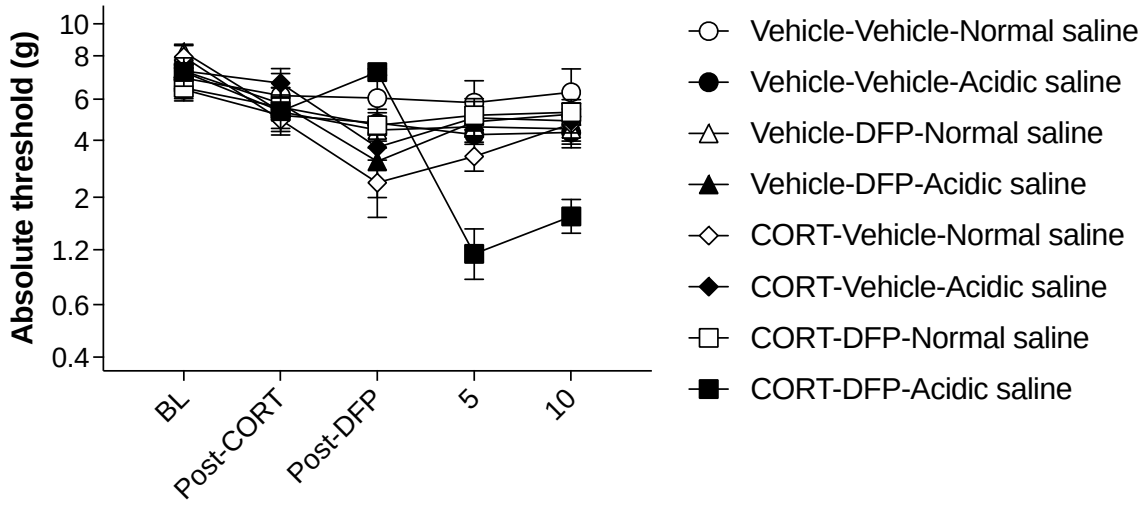

Figure 4. Baseline nociceptive measures were performed, and rats received either CORT or vehicle control in the drinking water for 7 days. On day 7, rats received a single subcutaneous injection of DFP or vehicle. Rats were allowed to recover from the acute DFP toxicity for 1 week and, after pre-injection behavioral testing, received a single, unilateral IM injection of acidic saline (pH 4) or normal saline (pH 7) into the gastrocnemius muscle. Von Frey testing of the ipsilateral paw followed on days 5, and 10 after acidic/normal saline injection.

Thermal hyperalgesia, as measured by the Hargreaves test, did not develop after any combination of treatments (**Figure 5**).

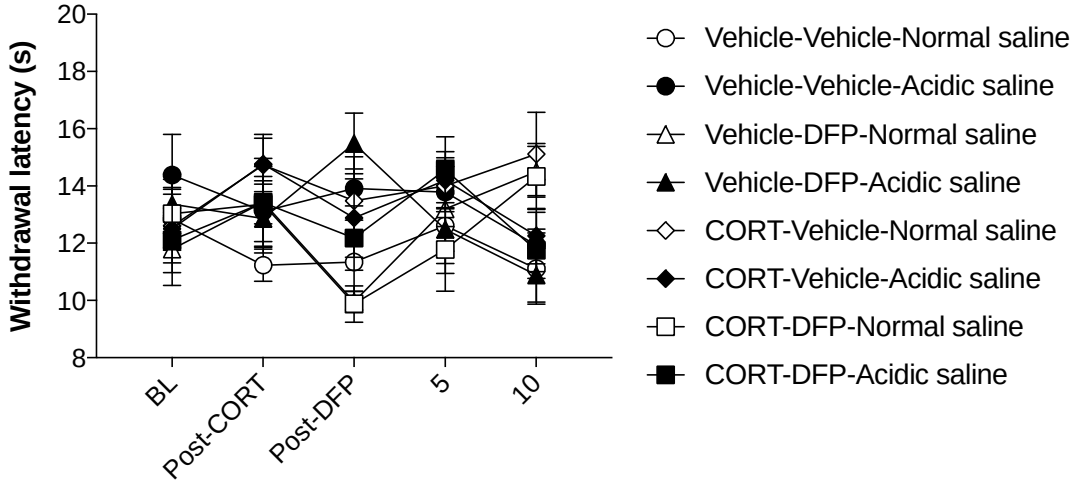

Figure 5. Baseline nociceptive measures were performed, and rats received either CORT or vehicle control in the drinking water for 7 days. On day 7, rats received a single subcutaneous injection of DFP or vehicle. Rats were allowed to recover from the acute DFP toxicity for 1 week and, after pre-injection behavioral testing, received a single, unilateral IM injection of acidic saline (pH 4) or normal saline (pH 7) into the gastrocnemius muscle. Hargreaves testing of the ipsilateral paw followed on days 5 and 10 after acidic/normal saline injection.

Results from the mechanical conflict-avoidance task are pending.

<u>Major Task 4</u>: Acidic/normal saline administered <u>14</u> days after final DFP injection for tissue analyses. IN PROGRESS

The purpose of this task is to determine whether a model of GWI (CORT+DFP) creates neuroinflammation in the pain neuraxis. This task is in progress. Animals have been treated according to the experimental plan, and tissues were collected at days 5 and 20 post acidic saline (n=5-8 per group). We are dosing DFP at the LD25, and some rats lost more than 20% of their starting body weight, resulting in euthanasia prior to the tissue collection timepoint. Thus, we will need to run additional animals to adequately power some experimental groups.

We have completed the LC-MS analysis of spinal cord and DRG samples. In **Figure 6**, we show that key sphingolipids are not modified in the GWI model. This result influences Major Task 6, as it indicates that S1PR1 antagonists (FTY720) will not be an effective treatment.

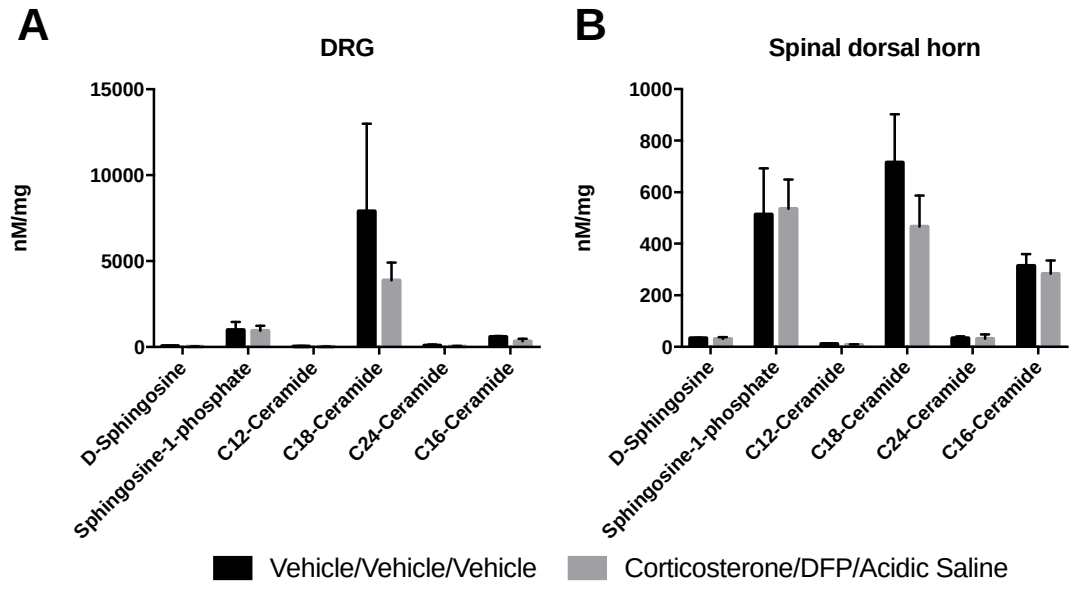

Figure 6. Sphingomyelin levels in A) DRG and B) spinal dorsal horn.

The remaining biochemical analyses are pending and will be completed in Year 3.

<u>Major Task 5</u>: Acidic/normal saline administered <u>180</u> days after final DFP injection for tissue analyses. IN PROGRESS

The purpose of this task is to determine whether a model of GWI (CORT+DFP) creates neuroinflammation in the pain neuraxis. This task is in progress. Animals have been treated according to the experimental plan, and tissues are scheduled for collection within the month (n=4-8 per group). We are dosing DFP at the LD25, and some rats lost more than 20% of their

starting body weight, resulting in euthanasia prior to the tissue collection timepoint. Thus, we will need to run additional animals to adequately power some experimental groups. Biochemical analyses will be completed in Year 3.

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.

The research personnel listed on this project have had extensive training and professional development opportunities. These include the technical aspects of this project, like the von Frey test, Hargreaves test, Mechanical Conflict-Avoidance System, necropsy, and Western blotting. Professional development opportunities include poster presentations at conferences, and oral presentations at lab meetings.

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.

This project and the latest results were presented at the Society for Neuroscience Annual meeting in Washington DC in Novermber 2017, and at the International Association for the Study of Pain in Boston in September 2018.

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

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

Major Tasks 3-7 will be completed within the next reporting period. In addition, we will begin preparation a manuscript to report the results.

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.

Nothing to report.

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.

The PI experienced significant delays in instituting his behavioral research within the Department of Critical Care Research. These included the absence of space required to reliably conduct behavioral experiments. As these problems were not resolved after 18 months, the PI transferred to the Department of Symptom Research. This transfer also caused another delay, as the PI also had to relocate his Laboratory to the Department of Symptom Research, which impacted analysis of tisses.

However, the PI has now been assigned 2 rooms with sufficient space to perform behavioral experiments, and his laboratory is running again.

It should also be noted that the PI is expected to move his laboratory to a new building in Spring/Summer 2019; this is a scheduled move for the Department of Symptom Research. Several weeks of down-time is expected.

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

N/A

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: 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.*

Lacagnina MJ, Fabisiak TJ, Sullivan K, O'Callaghan J, Watkins LR, Grace PM (2017). A neuroimmune basis for chronic pain in a rat model of gulf war illness. Society for Neuroscience, 2017: Washington DC.

Grace PM, Lacagnina MJ, Lorca S, O'Callaghan JP (2018). Neuroimmune signaling in a rat model of Gulf War Illness pain. IASP World Congress, 2018: Boston, MA.

Odem MA, Lacagnina MJ, Katzen SL, Li J, Wicks KC, Spence EA, Grace PM, Walters ET. A novel operant mechanical conflict assay reveals persistent post-surgical pain-avoidance behavior in sham surgeries for central and peripheral neural injuries. (in review).

- **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.*

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."

Name:	*Peter Grace*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*orcid.org/0000-0002-8999-1220*
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Grace was responsible for overall project management, oversight of all experimental designs and execution, and presentation of the results at national forums. Dr. Grace also trained personnel on experimental procedures.*

Name:	*Michael Lacagnina*
Project Role:	*Postdoctoral Researcher*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Lacagnina conducted experiments, including drug preparation, injections, behavioral assessments, tissue collection, and performed data analysis. His managerial responsibilities included procuring materials required to complete the project.*

Name:	*Jiahe Li*
Project Role:	*Postdoctoral Researcher*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Li conducted experiments, including drug preparation, injections, behavioral assessments, tissue collection, and performed data analysis.*

Name:	*Sabina Lorca*
Project Role:	*Research Assistant II*
Researcher Identifier (e.g. ORCID ID):	*n/a*
Nearest person month worked:	*9*
Contribution to Project:	*Ms. Lorca's responsibilities included drug preparation, injections, and tissue collection. Managerial responsibilities included procuring*

materials required to complete the project and performing daily animal monitoring.

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.

Nothing to report.

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.

Nothing to report.