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TITLE: Suppression of GWVI Toxin-Activated Microglia and Pathologies by DREADD

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Approximately one third of Veterans who served in the Gulf War later developed a chronic multi-symptom illness known as Gulf War Illness (GWI). While the exact cause is unknown, it is believed that persistent exposure to environmental toxins such as pesticides and chemical warfare agents may have interacted with combat-related stress to produce lasting neurological and psychiatric complications among this Veteran population. Neuroinflammation has been increasingly linked with psychiatric and neurological disorders and may play a role in GWI pathology. Microglia are a key mediator of neuroinflammation and the underlying goal of this project is to test the hypothesis that microglial activation acts as a causal factor to produce cognitive and psychiatric disturbances in a mouse model of GWI. In particular, this project will utilized novel Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology to inactivate microglia in our mouse model of GWI.

15. SUBJECT TERMS NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	12	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

Gulf War Illness represents The overall goal of this DOD funded research study is to develop a novel mouse model of Gulf War Illness (GWI) and test the hypothesis that microglia are a key mediator of GWI neuropathology. In pursuit of these goals, we are utilizing a Cx3Cr1-dependent designer receptor exclusively activated by a designer drug (DREADD) for the suppression of microglia in mice. In particular, we are using this mouse to test the hypothesis that microglial activation mediates neuroinflammation and behavioral abnormalities after exposure to permethrin and stress.

2. KEYWORDS

Gulf War Illness, microglia, permethrin, stress, pyrethroid, DREADD, learning and memory, depression, anxiety

3. ACCOMPLISHMENTS

a. Major Goals of the Project:

Finalize Breeding of DREADD Mice (Year 1)

-Generate a Breeding colony to produce sufficient numbers of DREADD mice necessary for all experiments.

Validate DREADD mediated microglial activation with LPS (Year 1)

-Use LPS administration to induce microglial activation

-Use inactivate microglia via administration of clozapine-n-oxide to validate technique.

Immunohistochemistry of microglia (Year 2)

-Quantify microglial activation in mouse model of GWI and suppression.

Animal Behavior Assesments (psychiatric) (Year 2)

-Determine if microglial suppression in DREADD mice prevents spatial memory impairments in GWI mouse model.

Animal Behavior Assessments (cognitive) (Year 3)

-Determine if microglial suppression in DREADD mice prevents spatial memory impairments in GWI mouse model.

Brain/Plasma Cytokine and FACS of Neuroimmune Cells (Year 3)

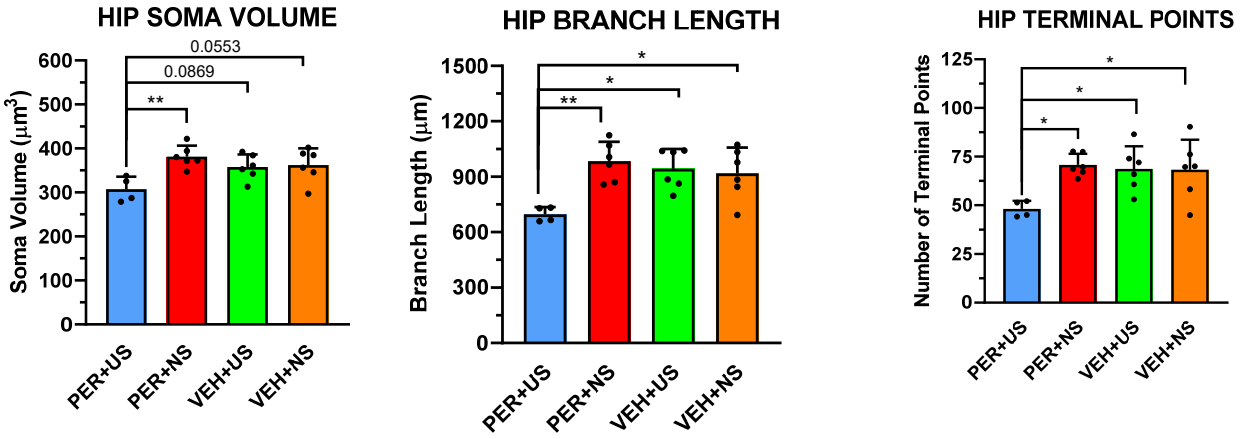
-Collect brains and plamsa from mice exposed to permethrin and stress to analyze immune cell populations via FACs

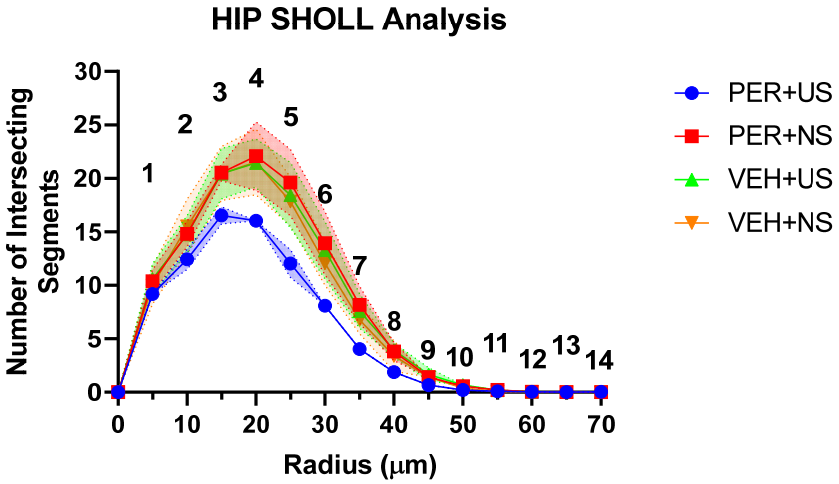
Peripheral Leukocye Immunophenotyping (Year 3)

-Determine if microglial suppression in DREADD mice affects peripheral immune cell phenotypes.

b. Accomplishments Under These goals

During the previous reporting period we validated a novel model of Gulf War Illness in which mice were chronically exposed to permethrin for 14 days followed by 7 days of unpredictable stress. We determined that this model was sufficient to induce a depressive behavioral phenotype as measured via forced swim test. During this reporting period we determined that our model is sufficient to induce changes in microglial activation (figure 1). An additional goal of the proposed studies was to generate a colony of Cx3Cr1-dependent DREADD mice for suppression of microglia. We have successfully generated and maintained this mouse colony. During this reporting period we also sought to validate the utility of this mouse line for blunting neuroinflammation-induced behavioral changes in mice. To accomplish these goals we utilized lipopolysaccharide (LPS) to stimulate inflammation and behavioral changes in mice. Surprisingly, these studies revealed that LPS was not sufficient to induce depressive or anxiety-like behaviors in mice (figure 2). We then performed additional biochemical studies to determine if our Cx3Cr1-dependent DREADD line was sufficient to suppress LPS-induced changes in the secretion of pro-IL-1β and Il-1β across multiple brain regions (figures 3 and 4). Finally, we sought to determine if our Cx3Cr1-dependent DREADD line was sufficient to suppress the behavioral phenotype observed in our GWI model (figure 5). Excitingly, the use of our novel transgenic mouse line was sufficient to suppress the depressive phenotype observed in our GWI model (figure 5). Additionally, we are currently in the process of performing fluorescence activated cell sorting (FACS) using blood and brain tissue from GWI mice. Specifically, we are measuring changes in CD45, CD11b, TLR4, CD14, RAGE, CD86, MHC-II, or CD3, Ly6C, CD19.





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AAA VS CCC	NS	0.0698	***	****	****	****	**	NS	NS	NS	NS	NS	NS	NS
AAA VS DDD	NS	**	***	****	****	***	*	NS	NS	NS	NS	NS	NS	NS
BBB VS CCC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
BBB VS DDD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CCC VS DDD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Figure 1. Exposure to permethrin followed by mild stress induced microglial activation in the hippocampus as determined via measurement of soma volume, branch length, terminal points, and sholl analysis

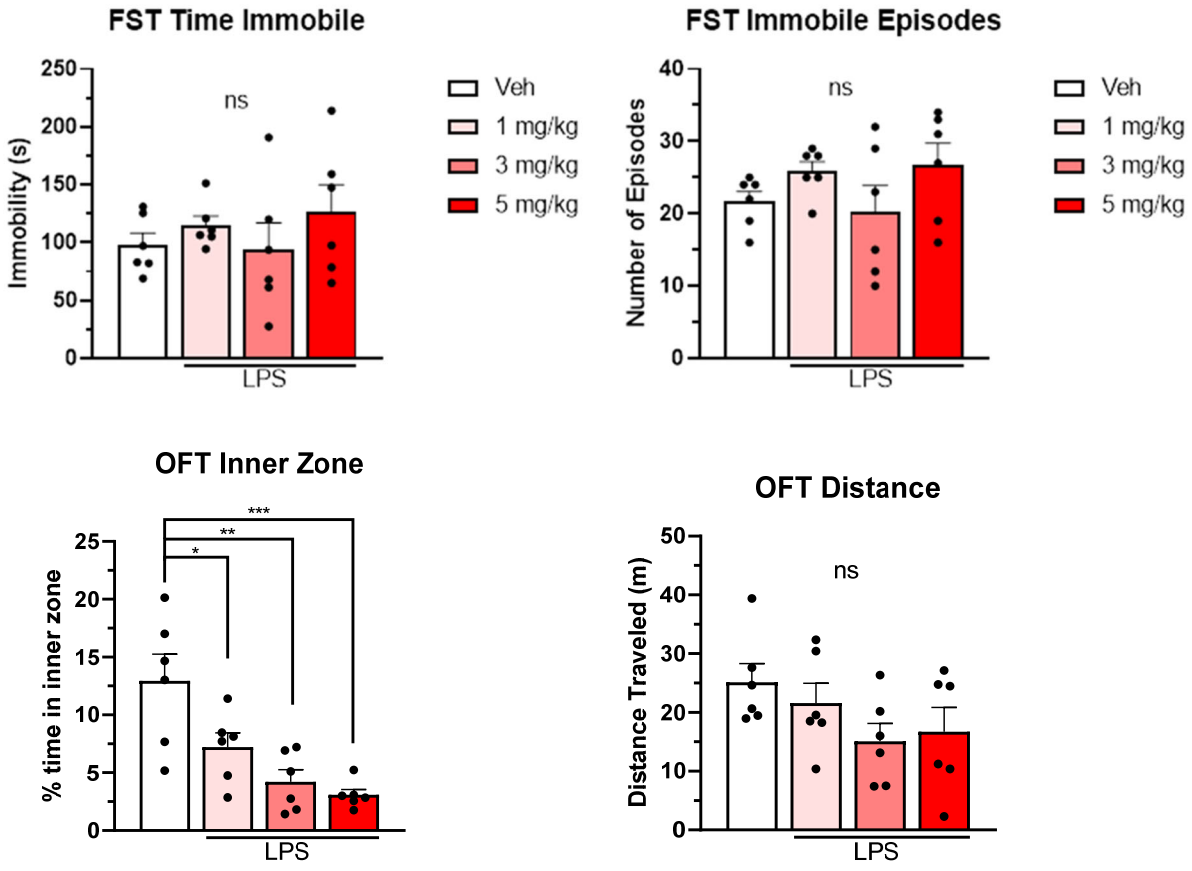

Figure 2. Administration of lipopolysaccharide (LPS) did not induce depressive or anxiety like behaviors in the forced swim test (FST) or open field test, respectively. LPS did produce a dose-dependent increase in the amount of time spent in the inner zone of the OFT, however this is opposite to what would be expected for anxiety-like behaviors.

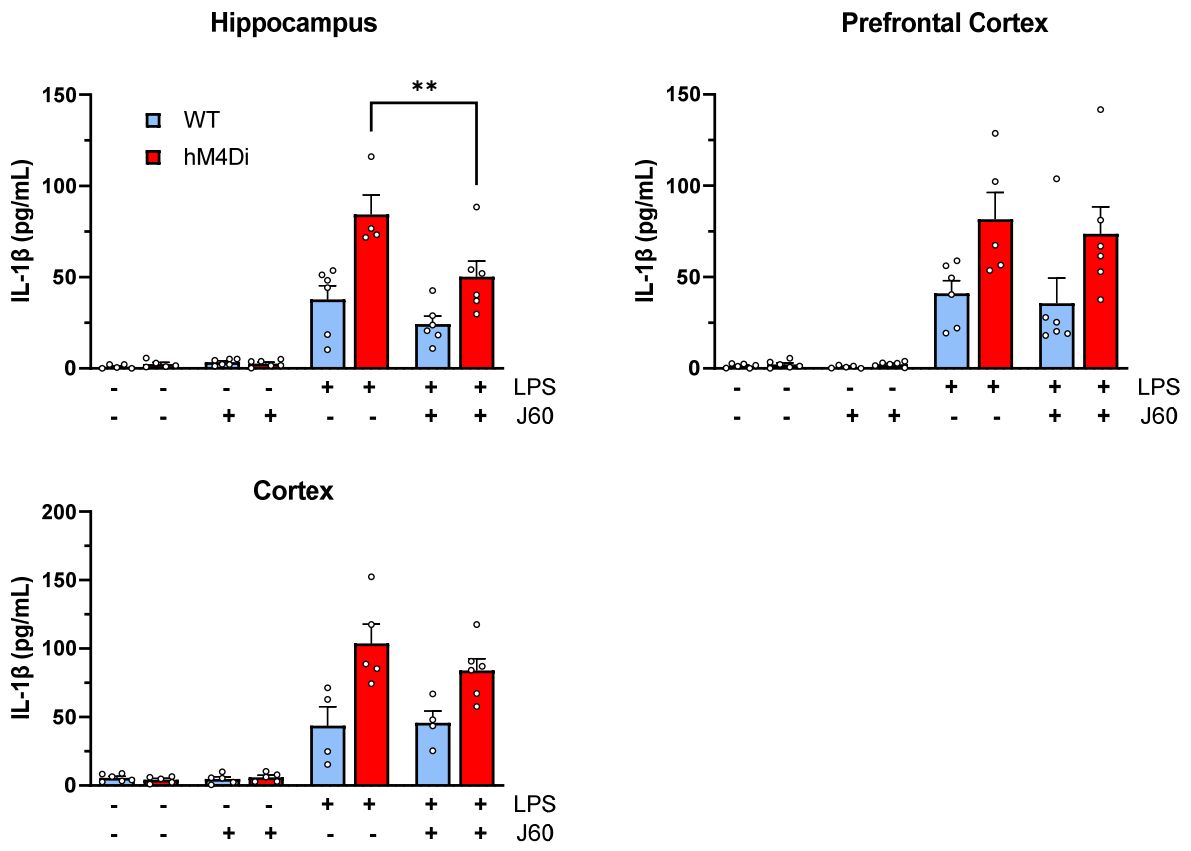

Figure 3. Activation of the DREADD inhibitory receptor hM4Di via the specific ligand JHU37160 (J60) significantly suppressed levels of IL-1β in the hippocampus, but not the prefrontal cortex or cortex as determined via ELISA.

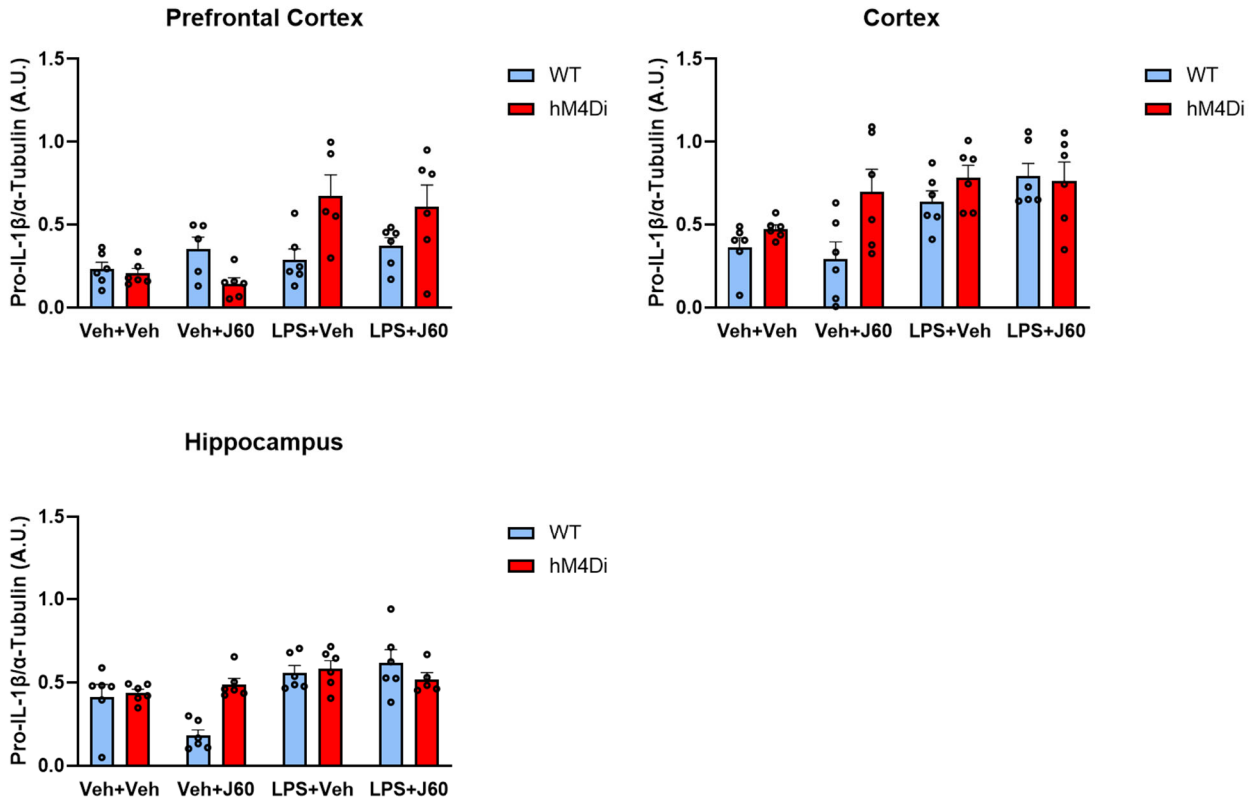

Figure 4. Activation of the DREADD inhibitory receptor hM4Di via the specific ligand JHU37160 (J60) did not suppress levels of the uncleaved PRO-IL-1β in the hippocampus, prefrontal cortex or cortex as determined via Western Blot.

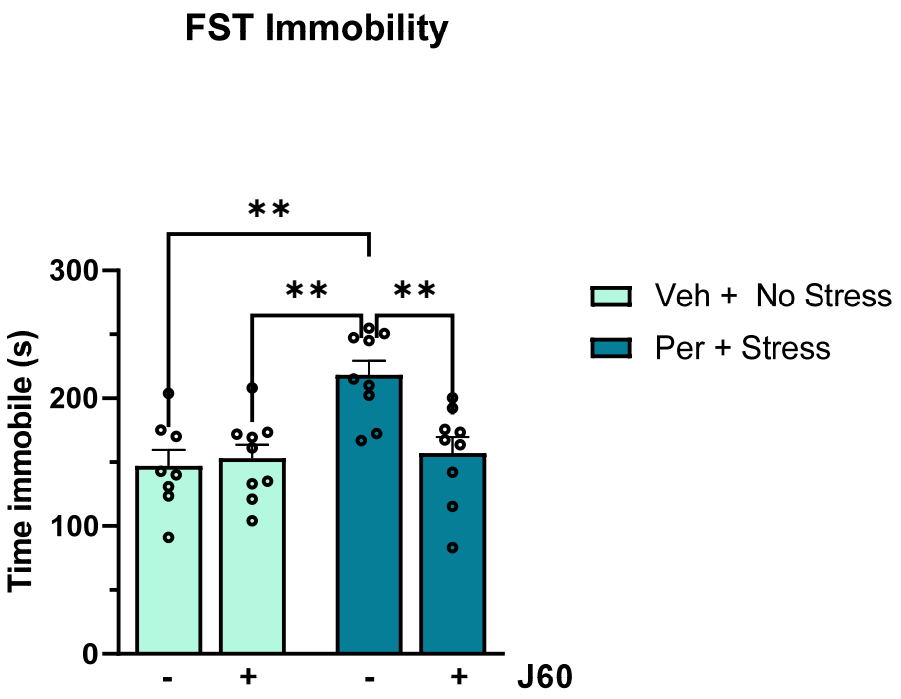

Figure 5. Behavioral changes in mouse model of GWI. Activation of the DREADD inhibitory receptor hM4Di via the specific ligand JHU37160 (J60) was sufficient to block the depressive phenotype previously observed after chronic treatment with permethrin followed by stress.

c. Opportunities for Professional Development

Nothing to report.

d. Dissemination of Information to Communities of Interest

Nothing to report.

e. Plan for next reporting period

As we move into the next reporting period we plan to examine changes in the proportion of peripheral plasma T helper cells, then determine if suppression of microglia prevents such changes. Specifically, we will collect blood from GWI mice and isolate plasma to characterize immunophenotypes of blood lymphocyte cells using multiparametric FACS. This will allow for characterization of T effector cells and T helper populations in the plasma, respectively. Additionally, we will continue to perform behavioral and immunohistochemical analysis of additional cohorts of animals.

4. **Impact**
    a. **What was the impact on the development of the principal discipline(s) of the project?**
    Nothing to Report

    b. **What was the impact on other disciplines?**
    Nothing to Report

    c. **What was the impact on technology transfer?**
    Nothing to Report

    d. **What was the impact on society beyond science and technology?**
    Nothing to Report

5. **CHANGES/PROBLEMS**
    a. **Changes in approach and reasons for change**
    We initially planned to conduct mouse behavioral studies to assess spatial learning and memory during the first year and anxiety and depressive behaviors during the second year. We instead conducted anxiety and depressive behaviors during the first year and plan to measure spatial memory during the second year. This was because the behavioral tasks for depression and anxiety (forced swim and open field) were easier to perform while adhering to social distancing requirements during the COVID-19 pandemic.

    b. **Actual or anticipated problems or delays and actions or plans to resolve them**
    Nothing to Report

    c. **Changes that had a significant impact on expenditures**
    Nothing to Report
    Significant changes in use or care of human subjects
    Nothing to Report

    d. **Significant changes in use or care of vertebrate animals.**
    Nothing to Report

    e. **Significant changes in use of biohazards and/or select agents**
    Nothing to Report

6. **PRODUCTS**
    a. **Journal Publications**
    Nothing to Report

    b. **Books or other non-periodical, one-time publications**
    Nothing to Report

c. **Other publications, conference papers, and presentations**
Noting to report.

d. **Website(s) or other Internet site(s)**
Nothing to report.

e. **Technologies or techniques**
Nothing to report

f. **Inventions, patent applications, and/or licenses**
Nothing to report
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g. **Other Products**
Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **Individuals who have worked on the project**

i. **Dr. Sean X Naughton, PhD**
Role: Post-Doctoral Fellow
Contribution: Responsible for overall design of the project and execution of experiments.
Funding Support: 10%

ii. **Kyle Trageser, BS**
Role: Research Assistant
Contribution: Assisting in the execution of experiments.
Funding Support: 5%

iii. **Dr. Giulio Pasinetti, MD PhD**
Role: Principle Investigator
Contribution: Overall Experimental Design.
Funding Support: 15%

b. **Changes in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period**
Nothing to report

c. **Other Organizations involved as partners**
Nothing to Report

8. Special Reporting Requirements
Nothing to Report

9. Appendices
Nothing to Report