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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
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1. INTRODUCTION

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 Assessments (psychiatric) (Year 2)

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

Peripheral and Neuroimmune Interaction Studies (Year 3)

-Collect brains and blood from mice exposed to permethrin and stress to analyze immune cell populations via transcriptomic studies and single cell RNA sequencing.

Peripheral Immune Studies (Year 4)

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

Animal Behavior Assessments (cognitive) (Year 4)

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

b. Accomplishments Under These goals

During the first year we validated a novel model of Gulf War Illness (GWI) 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 the second year we determined that our model is sufficient to induce changes in microglial activation. 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 the third year we assessed the utility of this mouse line for blunting neuroinflammation-induced behavioral changes in our mouse model of GWI. We determined that our Cx3Cr1-dependent DREADD line was sufficient to suppress the behavioral phenotype observed in our GWI model.

During the current reporting period we performed additional studies to understand the role of neuro-immune interactions. Specifically, we analyzed blood from mice exposed to permethrin for 14 days followed by 7 days of unpredictable stress (our GWI model). We analyzed blood transcriptomic changes via a specific panel of immunological-relevant probes. We observed a number of immunological changes in the blood of mice exposed to our treatment. In particular, mice exposed to permethrin followed by unpredictable stress exhibited decreases in T-Cell receptor signaling, TGF-β signaling, and Th2 differentiation (Figure 1). When comparing specific changes in differentially expressed genes we encountered a number of interesting, but not statistically significant changes when comparing mice exposed to permethrin followed by unpredictable stress with mice treated with Vehicle and No Stress (Figure 2), as well as Permethrin without stress when compared to Vehicle without stress (figure 3) and vehicle with stress compared to vehicle without stress (figure 4). We next performed single-nuclei RNA sequencing to identify differentially expressed genes in microglia and other brain cell populations in response to our treatment. We generated a single-nuclei atlas representing the detected populations of various cell types based on their gene expression profiles (figure 5). We are currently using this atlas to conduct additional bioinformatic analysis to quantify differentially expressed genes in each of the relevant cell populations.

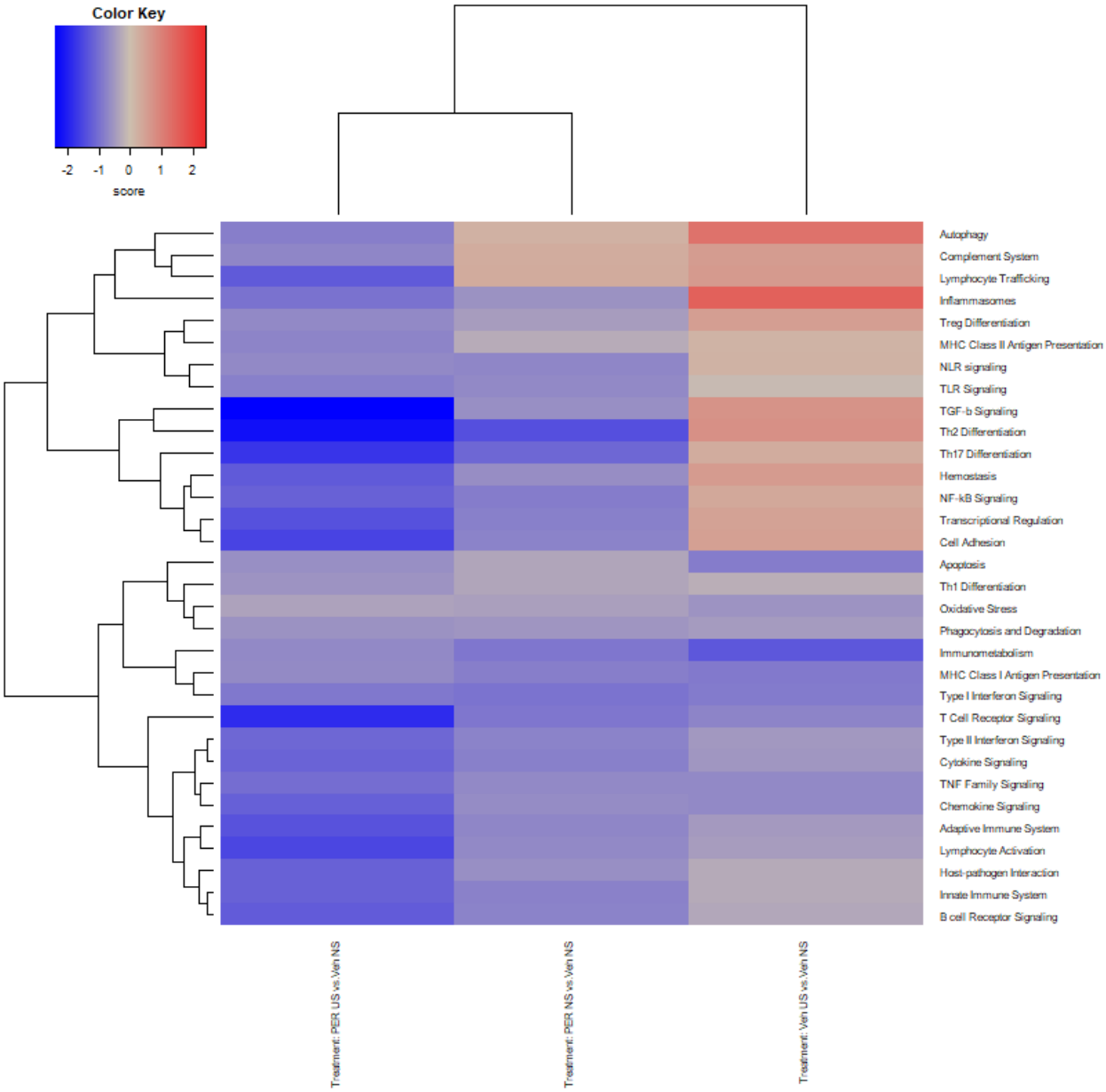

Figure 1. **Peripheral Blood Transcriptomic Changes.** Exposure to permethrin followed by unpredictable stress (PER US) lead to changes in gene expression associated with T-Cell receptor signaling, TGF-β signaling, and Th2 differentiation. Exposure to permethrin (PER NS) alone was associated with only mild changes in gene expression. Exposure to stress alone (VEH US) was associated with changes in genes relevant to inflammasome activation, autophagy, and peripheral immune cell signaling.

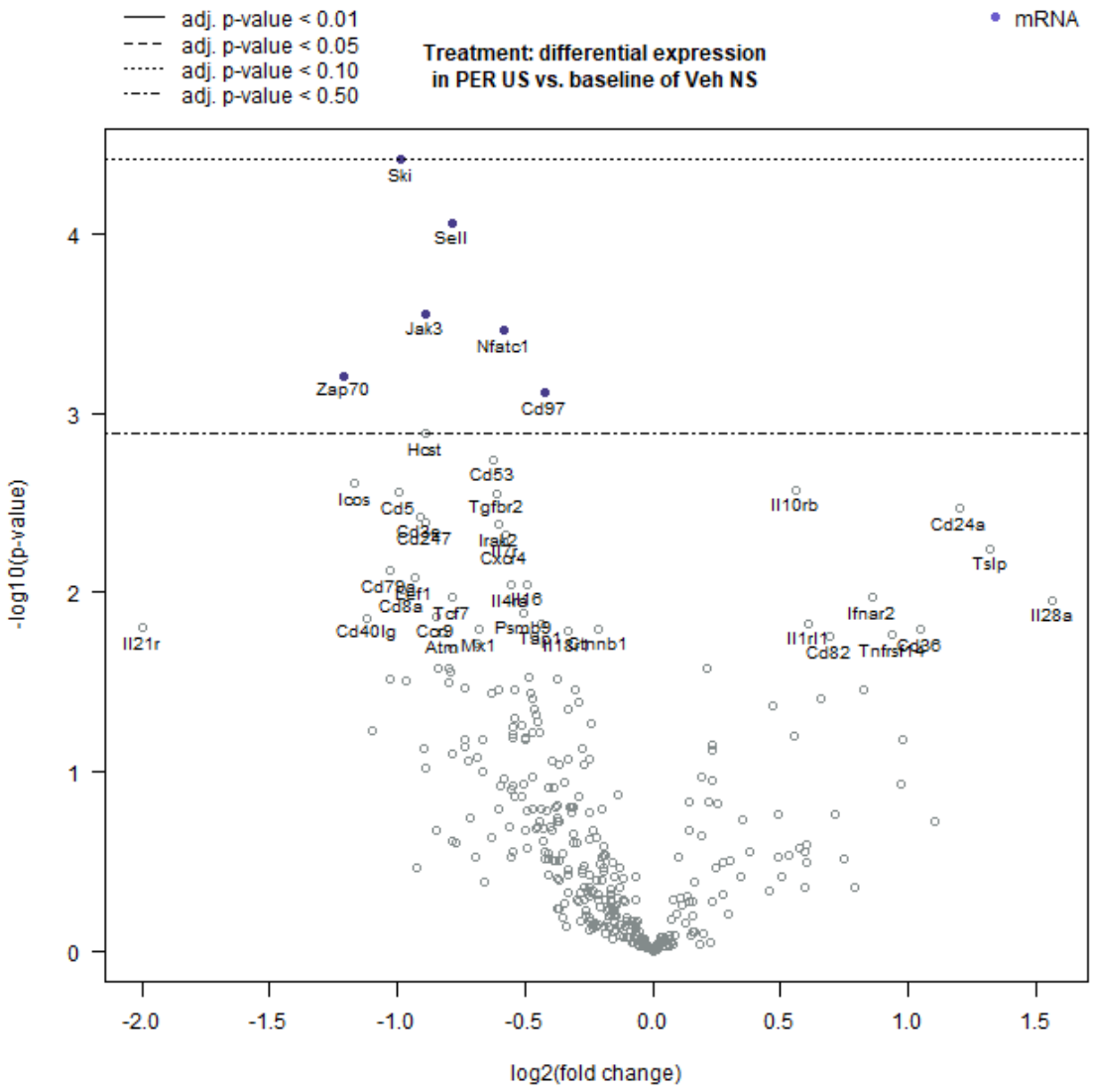

Figure 2. Genes effected by Permethin and Unpredictable Stress (PER US). We observed trending changes in the expression of Ski, Sell, Jak3, Zap70, Nfatc1, and Cd97 after exposure to permethrin followed by stress.

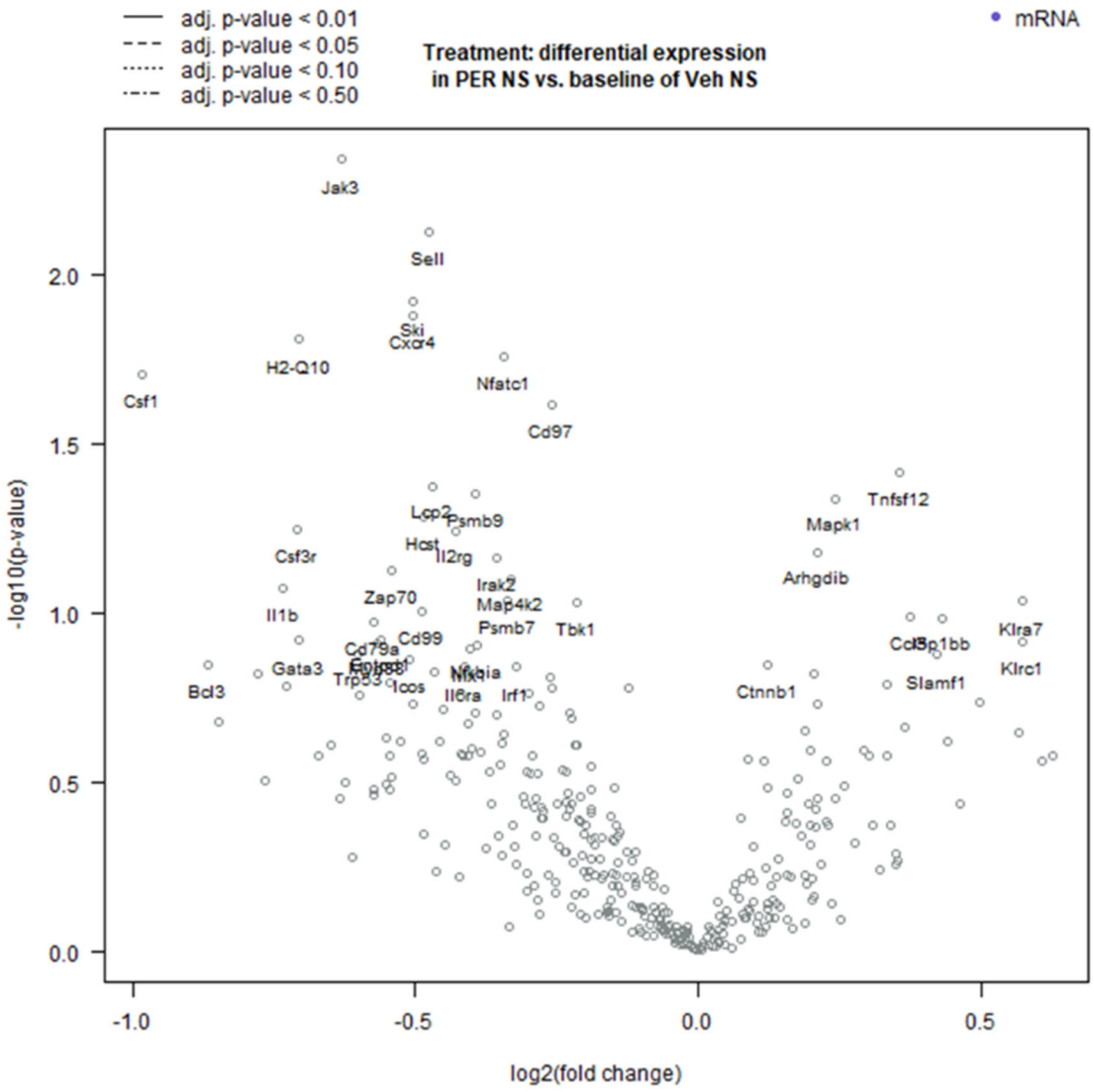

Figure 3. Genes effected by Permethin Alone (PER NS). Exposure to permethrin without subsequent stress exposure led to slight, but non-significant decreases in expression of the genes Jak3 and Sell.

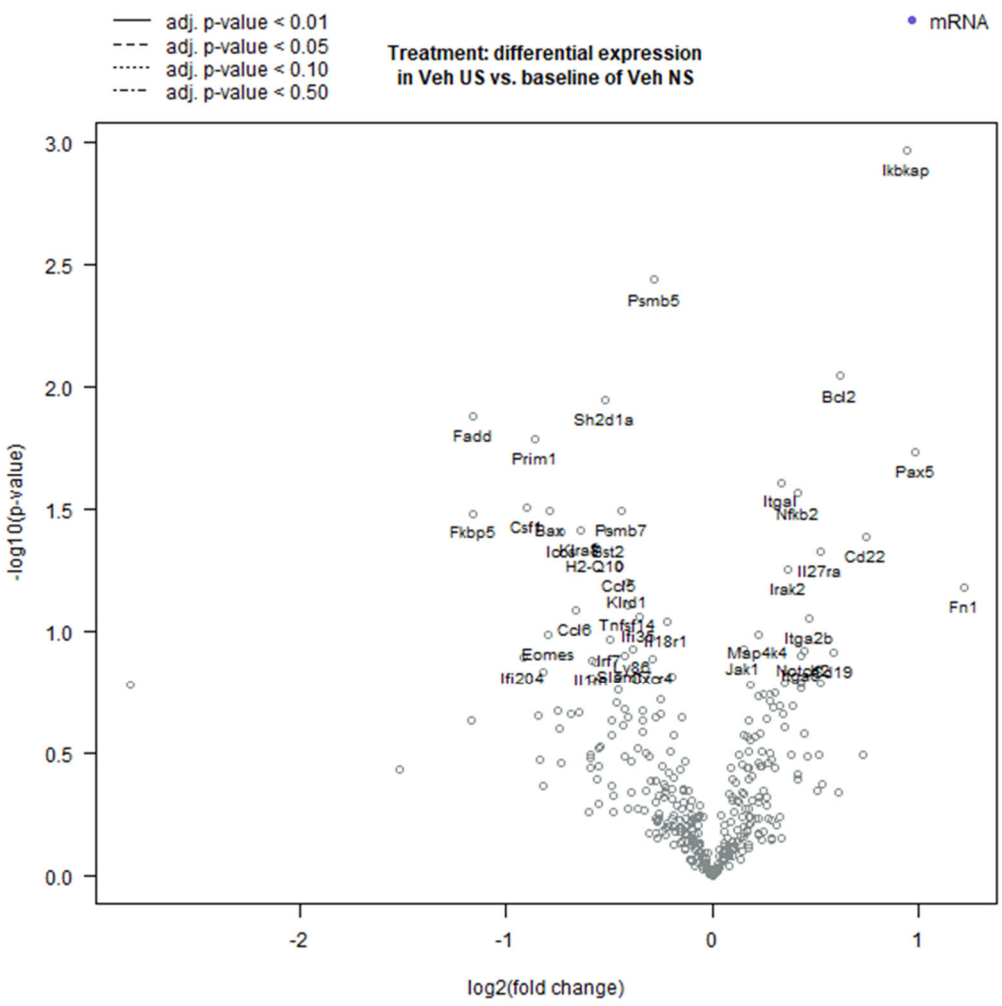

Figure 4. Differentially Expressed Genes affected by Stress Alone (VEH US). Exposure to stress alone resulted in a mild, but non-significant, increase in the expression of Ikbkap.

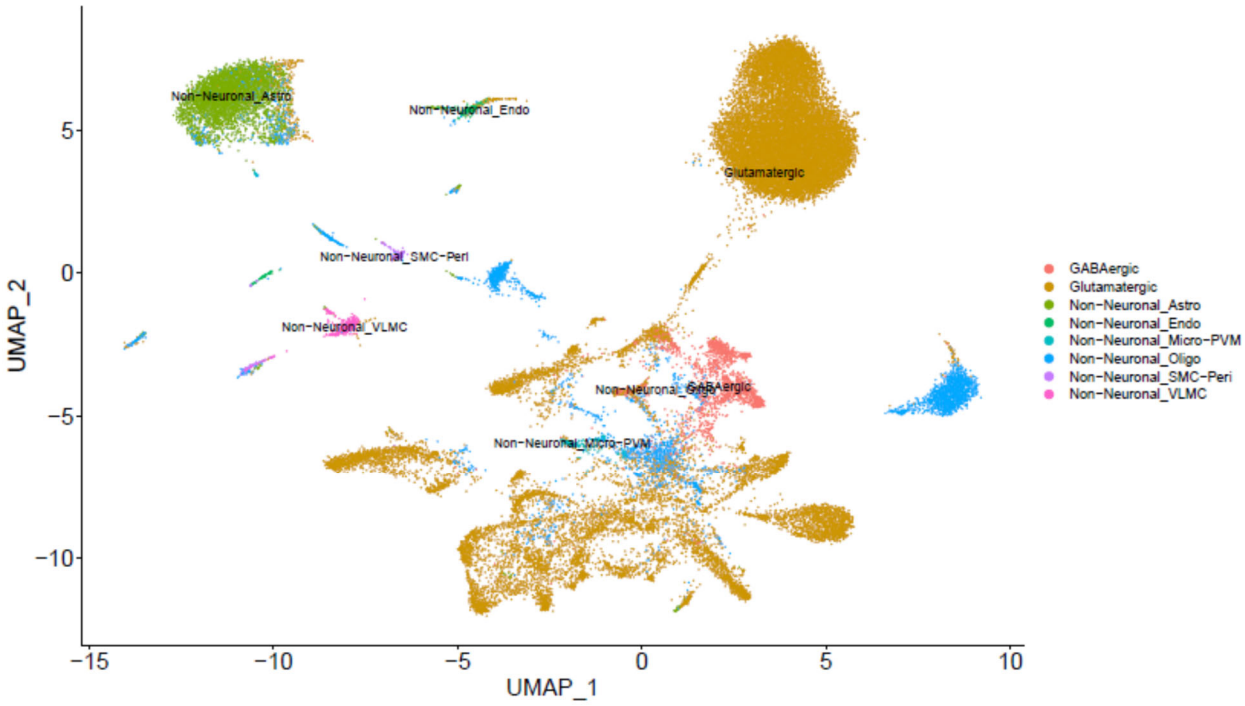

Figure 5. Single-Nuclei RNA sequencing atlas of cell types affected by our GWI treatment model. **S**ingle-nuclei RNA sequencing atlas, which will allow for further investigation of cell-type specific changes in gene expression after exposure to permethrin and stress.

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 conduct a final series of behavioral studies to determine if exposure to permethrin followed by stress is capable of inducing changes in learning and memory, as measured via Morris water maze. Additionally, we plan to evaluate whether or not suppression of microglia in our DREADD model results in any changes in peripheral cytokine expression or changes in peripheral immunological markers.

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 perform our neuroimmune analysis via FACS sorting, we instead made use of newer, more advanced, single-nuclei RNA sequencing technology.

    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