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14. ABSTRACT

Post-traumatic Stress Disorder (PTSD) is common amongst combat exposed military personnel, but its pathophysiology is poorly understood. Current approved treatments, targeting the serotonergic system, fail to fully reduce the burden of this disease and do not account for dysfunction in other mechanistic pathways including immune dysregulation and inflammation. In this proposal, we focus on an understudied signaling pathway, the Aryl Hydrocarbon Receptor (AhR) pathway, in the pathophysiology of PTSD and associated psychological conditions after combat exposure. This pathway has been shown to modulate a wide variety of physiological processes including immune function and changes in the activation of this pathway have been identified in diseases with an inflammatory component. Furthermore, studies in MDD have demonstrated that ligands for these receptors are altered, specifically demonstrating that those with pro-inflammatory activity (kynurenine) are increased and those with an anti-inflammatory activity (Indoles) are reduced. In this proposal, we investigate not only overall AhR signaling, but also study known AhR ligands which have been implicated in psychiatric disease and their role in the development of PTSD. Plasma samples for assay have recently been obtained and we have been able to demonstrate the ability of our cell based assay to reliably detect kynurenine metabolites of tryptophan. We are now optimizing the assay to detect AhR ligands in the plasma. Additionally, targeted metabolomics for kynurenine and indole metabolites of tryptophan will be completed shortly and will allow us to determine the physiological concentrations of these metabolites.

15. SUBJECT TERMS
None listed.

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INTRODUCTION:

PTSD is one of the major "signature injuries" of the Iraqi and Afghanistan Wars, and is associated with much personal suffering, poor mental and physical health, and economic cost, and in many cases, depression, shame and self-blame and diminished work performance and quality of personal relationships. While the environmental precipitants of PTSD are clear, the biological factors placing individuals at risk for developing PTSD and those that underlie the progression of PTSD are poorly understood. This lack of adequate understanding of the underlying biology of PTSD largely contributes to the poor rate of remission with current drug treatments; only 30% of treated individuals fully remit with standard medication treatment. This highlights the need to identify new biological mechanisms that underlie PTSD and associated co-occurring conditions such as depression and traumatic brain injury. The identification of these mechanisms should lead to the development of better risk prediction, improved diagnoses, and more effective treatment strategies. One candidate mechanism in the biology of PTSD involves a receptor in certain cells, especially immune cells, that plays an important role in many inflammatory medical conditions, called the Aryl Hydrocarbon Receptor (AhR). We propose that altered activity at this receptor plays a significant role in PTSD, but this has never previously been investigated, despite PTSD having a strong inflammatory aspect. In this proposal, we aim to understand the role of the Aryl Hydrocarbon Receptor (AhR) signaling pathways in the biology of PTSD and associated co-occurring conditions.

KEYWORDS:

Post-traumatic Stress Disorder

Aryl Hydrocarbon Receptor

Kynurenine

Indoles

ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Specific Aims/Hypotheses

Aim 1: Identify net alterations in activation of the Aryl Hydrocarbon Receptor (AhR) pathway in PTSD and associated psychological conditions using an *in vitro* cell-based reporter assay.

H1a: Combat PTSD is associated with increased activation of the AhR pathway compared to combat trauma-exposed non-PTSD controls

H1b: Amongst individuals with combat PTSD, greater activation of the AhR pathway is associated with greater severity of psychiatric symptoms

H1c: Greater activation of the AhR pathway prior to deployment-related trauma predicts development of combat PTSD post-deployment

H1d: Development of combat PTSD (from pre- to post-deployment) is associated with an increase in the activation of the AhR pathway

Aim 2: Identify alterations in the abundance of known Aryl Hydrocarbon Receptor (AhR) ligands in PTSD and associated psychological conditions.

H2a: Combat PTSD is associated with an increase in kynurenine-related ligands of the AhR and a decrease in indole-related ligands of the AhR

H2b: Amongst individuals with combat PTSD, an increase in kynurenine-related AhR ligands and a decrease in indole-related AhR ligands are associated with greater severity of psychiatric symptoms

H2c: Greater abundance of kynurenine-related ligands of the AhR and a lesser abundance in indole-related ligands of the AhR receptor pre-deployment predicts development of combat PTSD post-deployment

H2d: Development of combat PTSD is associated with an increase in kynurenine-related ligands of the AhR and a decrease in indole-related ligands of the AhR receptor

<u>Exploratory Aim 1</u>: Identify alterations in *in vitro* activation of the AhR pathway and in the abundance of AhR ligands in PTSD associated with co-morbid MDD or comorbid combat related TBI.

H1a: Combat PTSD +co-morbid MDD is associated with greater activation of the AhR pathway and an increase in kynurenine-related ligands of the AhR and a decrease in indole-related ligands of the AhR receptor, compared to combat PTSD positive individuals without MDD.

H1b: Combat PTSD +comorbid combat-related TBI is associated with greater activation of the AhR pathway and an increase in kynurenine-related AhR ligands and a decrease in indole-related AhR ligands, compared to individuals without comorbid combat related TBI, and is associated with psychiatric symptom severity.

- **What was accomplished under these goals?**

In support of our Aims, we have been working to identify and obtain an appropriate number of stored samples to run all assays. During this time, we have been able to secure the majority of samples with PTSD and trauma exposed controls we initially proposed. These samples are currently being processed in preparation for our measurement of kynurenine/indole metabolites of tryptohpan (Aim 2 and Exploratory Aim 1). In support of Aim 1 of our project, we have obtained the AhR reporter assay and have been working to optimize this assay for plasma samples. In order to ensure proper functioning of the reporter, we have been optimizing the assay using a positive control (Kynurenine) which will also be measured in Aim 1 (Figure 1)

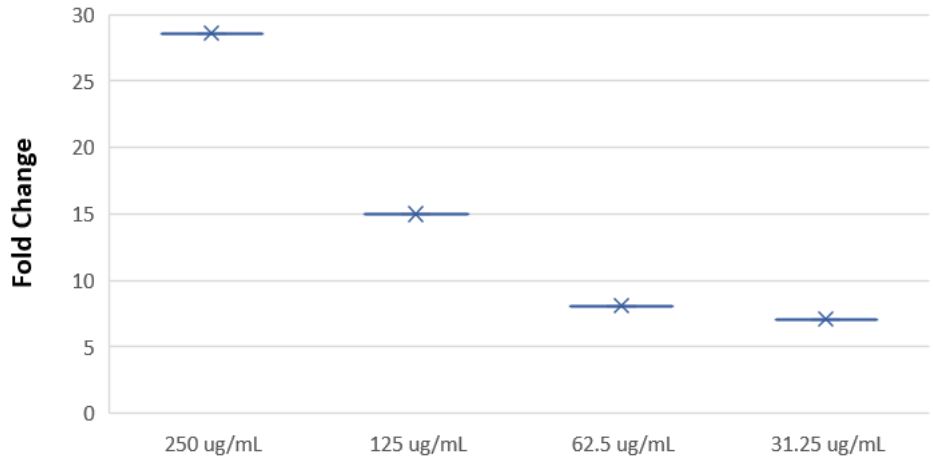

Figure 1. Detection of AhR agonist activity by purified Kynurenine. Purified kynurenine was incubated with the AhR reporter cell line for. Luciferase activity was measured and expressed as fold change relative to untreated cells. After optimization, we determine that 24 hours was the optimal timepoint at which to collect samples for luciferase measurement (18h, 48h, and 72h timepoints were also tested). We observed a dose dependent response where greater quantities of kynurenine were associated with heightened receptor activation which diminished with decreasing concentrations of kynurenine.

- **What opportunities for training and professional development has the project provided?**

Dr. Rampersaud, a new assistant professor in the department of Psychiatry and Behavioral Sciences, is carrying out portions of the work proposed in this grant. He has a significant interest in the role of tryptophan metabolites and AhR signaling in the pathogenesis of psychiatric illness. Additionally, an undergraduate research volunteers has also joined and is receiving training in statistical methods as well as laboratory methods and have been facilitating data collection and analysis. This experience has added to their professional skills and will strengthen their applications for graduate programs in biology and medicine.

- **How were the results disseminated to communities of interest?**

There are currently no results from the study to be disseminated.

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

In the next project period, we will continue to optimize conditions for the assay. Due to the greater availability of plasma (rather than serum), we are now identifying conditions to sallow for use of plasma in the reporter assay. Once this is completed, plasma samples from our PTSD participants and trauma exposed controls will be utilized in the assay. In support of Aim 2, we will also carry out targeted metabolomics for kynurenine/indole metabolites in collaboration with Dr. Lena Brundin at the Van Andel Institute. Upon completion of these assays, data analysis will be completed in the next project period.

IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**

  The potential findings from this study will indicate that there are differences in certain AhR ligands between healthy controls and individuals with PTSD, that may suggest novel cellular mechanisms that contribute to PTSD pathophysiology which may ultimately lead to novel therapeutic strategies. Furthermore, the use of our cell based reporter assay presents the possibility of a novel screening strategy for PTSD.

- **What was the impact on other disciplines?**

  Nothing to report

- **What was the impact on technology transfer?**

  Nothing to report

- **What was the impact on society beyond science and technology?**

  Nothing to report

CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
- **Actual or anticipated problems or delays and actions or plans to resolve them**
- **Changes that had a significant impact on expenditures**

Due to the lack of serum samples from our previously recruited active duty military participants, we must now use plasma samples, which will need to be optimized with our cell based AhR reporter assay. Given this change, we will have approximately 50 participants with PTSD with sufficient plasma and 50 combat exposed controls without PTSD (n = 90 serum samples had originally been proposed). If possible, we will include additional plasma from another cohort of participants with combat related PTSD to reach our original sample size (the number of samples available for our longitudinal assay is unchanged). Additionally, due to a small delay (which has

been resolved) in acquiring samples, we have not yet completed targeted metabolomics with our collaborators which will now take place in the next project period.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

  Nothing to report

- **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

PRODUCTS:

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

  - **Journal publications.** *Nothing to report*

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

- **Other publications, conference papers, and presentations.** *Nothing to report*

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

- **Technologies or techniques**
  Nothing to report

- **Inventions, patent applications, and/or licenses**
  Nothing to report.

- **Other Products**
  Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Owen Wolkowitz*

Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	*Dr. Wolkowitz is responsible for oversight of the project as well as data analysis and compilation of findings for publication*
Funding Support:	

Name:	*Sindy Mellon*
Project Role:	*Co-investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	*Dr. Mellon is responsible for oversight of the project as well as data analysis and compilation of findings for publication*
Funding Support:	

Name:	*Ryan Rampersaud*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1
Contribution to Project:	*Dr. Rampersaud will carry out laboratory-based portions of the study as well as data analysis and compiling findings for publication*
Funding Support:	

Name:	Gwyneth Wu
Project Role:	*Research Scientist*
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	5
Contribution to Project:	*Dr. Wu is responsible for data analysis, project management, and compiling findings for publication.*
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to report

- **What other organizations were involved as partners?**

  - **Organization Name:** Van Andel Institute
  - **Location of Organization:** Grand Rapids, Michigan
  - **Partner's contribution to the projec**t *(identify one or more)*
    - **Collaboration –** Dr. Lena Brundin's group is responsible for carrying out targeted metabolomics of Kynurenine and Indole metabolites of tryptophan.

5. **SPECIAL REPORTING REQUIREMENTS**

   Nothing to report

6. **APPENDICES:**

   None