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TITLE: Metabolomics: A Window for Understanding Long-Term Physical Consequences of Disturbed Sleep and Hypothalamic-Pituitary-Adrenal Function in Posttraumatic Stress

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14. ABSTRACT

Post-traumatic stress (PTS) is a common psychiatric condition that may result after combat exposure and can have a profound effect on sleep and physical health conditions, such as metabolic syndrome. Sleep disturbances may lead to alterations in stress response hormones of the hypothalamic-pituitary-adrenal (HPA) axis that may increase metabolic risk. Women may be at particularly high risk for these health concerns, given an increased prevalence of PTS and metabolic conditions in women compared to men. The purpose of this study is to identify biological mechanisms using a broad-based study of metabolomics that may explain differences in PTS, sleep disturbances, and metabolic risk in men and women. This broad approach can reveal circulating small molecules that affect cell and physiological function and will be used to identify biochemical pathways involved in PTS, sleep disturbances, and health. Metabolomic analysis will be performed on pre-collected plasma samples from a study that had a two-group cross-sectional design in which main comparisons were with medically healthy medication-free male and pre-menopausal female subjects with chronic PTS (N= 44) and trauma-exposed, age-matched controls (N= 44). Previously collected measures, including sleep EEG and metabolic markers (e.g., fasting glucose, insulin response to oral glucose tolerance test (OGTT)), fasting lipids, and leptin, will also be examined.

15. SUBJECT TERMS

Adrenocorticotropic hormone; Lipids; Hypothalamic-Pituitary-Adrenal; Kynurenine; Metabolomics; Neurosteroids; Posttraumatic Stress; Polyunsaturated Fatty Acids; Sex Differences; Sleep

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TABLE OF CONTENTS

1. INTRODUCTION:

Post-traumatic stress (PTS) is a common psychiatric condition that may result after combat exposure and can have a profound effect on sleep and physical health conditions, such as metabolic syndrome. Sleep disturbances may lead to alterations in stress response hormones of the hypothalamic-pituitary-adrenal (HPA) axis that may increase metabolic risk. Women may be at particularly high risk for these health concerns, given an increased prevalence of PTS and metabolic conditions in women compared to men. The purpose of this study is to identify biological mechanisms using a broad-based study of metabolomics that may explain differences in PTS, sleep disturbances, and metabolic risk in men and women. This broad approach can reveal circulating small molecules that affect cell and physiological function and will be used to identify biochemical pathways involved in PTS, sleep disturbances, and health. Metabolomic analysis will be performed on pre-collected plasma samples from a study that had a two-group cross-sectional design in which main comparisons were with medically healthy medication-free male and pre-menopausal female subjects with chronic PTS (N= 44) and trauma-exposed, age-matched controls (N= 44). Previously collected measures, including sleep EEG and metabolic markers (e.g., fasting glucose, insulin response to oral glucose tolerance test (OGTT)), fasting lipids, and leptin, will also be examined.

2. KEYWORDS:

Adrenocorticotropic hormone; Lipids; Hypothalamic-Pituitary-Adrenal; Kynurenine; Metabolomics; Neurosteroids; Posttraumatic Stress; Polyunsaturated Fatty Acids; Sex Differences; Sleep

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Study Goals	Timeline (months)	Percentage Complete
1. Study Start Up and Approvals	1-3	100%
2. Coordinate Study Staff for Sample Analysis	1-6	100%
3. Assay Biological Samples	7-20	95%
4. Data Analysis	20-28	50%
5. Finalize study requirements, prepare for future funding, and dissemination of findings	28-30	0%

What was accomplished under these goals?

The study started on September 30th 2016. This report describes accomplishments to date. All study start up activities were completed on schedule, including regulatory paperwork and approvals and hiring and coordination of study staff for sample analysis. Biological samples were organized, procedures for sample shipping and receiving was developed, a tracking system was created, and biological samples were shipped for processing. Three metabolite panels have been assayed to date, including primary amino acid metabolites, steroids and complex lipids. While initial group analyses have been performed by our group, specialized analyses (e.g., enrichment analysis with subsequent regression analyses) need to be performed to identify the subgroups of amino acid, steroid, and lipid metabolites that significantly differ by group with follow up on metabolic pathway analysis. Our detailed accomplishments to date include:

Major Task 1: Study Start Up and Approvals	**Timeline**
Subtask 1: Prepare regulatory documents and submit for IRB approval	Completed
Develop IRB application and other regulatory documents	Completed
Submit IRB application to UCSF IRB and obtain full committee review	Completed
Review by SFVAMC regulatory personnel	Completed
Review by HRPO	Completed
Prepare IRB reports for continuing review approvals	Annually
Milestone Achieved: IRB approval from UCSF, VA, and HRPO	Completed
Major Task 2: Coordinate Study Staff for Sample Analysis	**Timeline**
Subtask1: Hiring and Training of Study Staff	
Coordinate with NCIRE to prepare job description and advertisement	Completed
Interview research staff candidates	Completed
Coordinate with SFVAMC for candidate approval and required trainings	Completed
Training of research staff on study procedures and biospecimen storage, shipping, and receiving	Completed
Milestone Achieved: Research staff hired and trained	Completed
Subtask 2: Coordinate with laboratory personnel for sample shipments	
Contact staff at receiving laboratories	Completed
Develop procedures manual for sample shipping and receiving	Completed
Develop sample tracking system	Completed
Schedule batched shipments	Completed
Milestone Achieved: Sample shipment protocol established	Completed
Subtask 3: Build database for incoming data	
Work with Data Manager to establish data extraction protocol and build database	Completed
Establish logistical plan for data quality check	Completed
Milestone Achieved: Database built	Completed
Major Task 3: Assay Biological Samples	**Timeline**
Subtask 1: Ship stored samples to the receiving laboratory and acquire data	
Package and ship stored samples to UC Davis	Completed
Milestone Achieved: 1st batch of samples shipped for assay	Completed
Milestone Achieved: Final batch of samples shipped for assay	Completed
Subtask 2: Receive data from laboratory	Complete
Milestone Achieved: All data acquired	Complete
*Milestone Achieved: All Assays complete but data is under review (*Output is currently under final review for ensuring completeness and integrity of metabolomics data. Any anomalies found may require a repeated assay).	In progress
Major Task 4: Data Analysis	**Timeline**
Subtask 1: Enter data and maintain database	Complete
Perform quality checks on incoming data	Complete
Enter all data and maintain database	Complete

Subtask 2: Aim 1: To ascertain the neurosteroid (including glucocorticoid) metabolite profile in plasma of male and female patients with PTSD, and in healthy controls	
Clean and process incoming data and prepare for analysis	Complete
Subtask 3: Aim 2: To ascertain the primary amino acid and lipid metabolite profiles in plasma of male and female PTSD patients, and in healthy controls	
Clean and process incoming data and prepare for analysis	Complete

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

The PI has participated in the following scientific meetings:
2016 American Academy of Neuropsychopharmacology Annual Meeting
2017 American Academy of Neuropsychopharmacology Annual Meeting
2017 Biological Psychiatry Annual Meeting
2018 VA PTSD Psychopharmacology Initiative
2018 VA NCPTSD Women Veteran's Health Summit

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

Plans until next reporting period:

1. The metabolomics output is currently under review by Dr. Fiehn. Any anomalies that he finds may require a repeated assay.
2. Continue data analysis tasks: Perform quality checks on incoming data. Coordinate with Data Management for monitoring data entry and quality. Work with Biostatistician to conduct analyses (Task 4).
3. Begin dissemination of study findings. Finalize study requirements. Prepare for future funding (Task 5).

Major Task 4: Data Analysis	**Timeline**
Subtask 1: Enter data and maintain database	
Continue to perform quality checks on incoming assay data	In progress
Subtask 2: Aim 1: To ascertain the neurosteroid (including glucocorticoid) metabolite profile in plasma of male and female patients with PTSD, and in healthy controls	
Clean and process incoming data and prepare for analysis	In progress
Coordinate with Data Management for monitoring data entry and quality	In progress
Work with Biostatistician to conduct analyses	In progress
Share output and findings with co-investigators	In progress
Milestone Achieved: Aim 1	In progress
Subtask 3: Aim 2: To ascertain the primary amino acid and lipid metabolite profiles in plasma of male and female PTSD patients, and in healthy controls	
Clean and process incoming data and prepare for analysis	In progress
Coordinate with Data Management for monitoring data entry and quality	In progress
Work with Biostatistician to conduct analyses	In progress
Share output and findings with co-investigators	In progress
Milestone Achieved: Aim 2	In progress
Subtask 4: Conduct detailed pathway analyses for the subset of metabolites that are different between PTS vs. control and males vs. females.	*26-28*
Work with Biostatistician to conduct pathway analyses	*26-28*
Milestone Achieved: Pathway analyses complete	*28*
Subtask 5: Correlate levels of PTS and/or sex-specific metabolites to pre-collected measures of HPA axis function, sleep EEG, triglycerides, blood glucose, and body fat content.	*27-28*
Work with Biostatistician to conduct correlate analyses	*26-28*
Milestone Achieved: Correlate analyses complete	*28*
Milestone Achieved: All data analyses complete	*27-28*
Milestone Achieved: Characterize the metabolomic profile associated with glucocorticoid regulation mediating sleep and metabolic disturbances associated with PTS	*27-28*
Milestone Achieved: Identify specific metabolites associated with PTS for future clinical trial	*27-28*
Major Task 5: Finalize study requirements, prepare for future funding, and dissemination of findings	**Timeline**
Subtask 1: Dissemination of findings (abstracts, presentation, publications, DOD)	In progress
Subtask 2: Prepare grant application for DOD or VA Merit Award funding for a clinical trial based on study findings	29-30
Subtask 3: Complete final report.	30
Milestone Achieved: Report results from data analyses	
Milestone Achieved: Submit grant proposal for clinical trial to examine changes in specific metabolites and related inflammatory and metabolic processes on limbic responses in an fMRI paradigm.	

4. IMPACT:

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

Nothing to report

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

5. CHANGES/PROBLEMS:

The initial analyses examining group and sex differences in the primary amino acids, lipid and steroid panels were performed by Dr. Ritu Roy (for metabolites) and Mr. Thomas Metzler (for correlate measures). Due to the complex nature of metabolite analyses, we have asked for additional statistical support by Dr. Oliver Fiehn, director of the West Coast Metabolomics Center at UC Davis. Dr. Fiehn will review the output from assays that have been completed and will inform us if there are any errors or anomalies that he detects. Any assays that are under question will need to be redone. Dr. Fiehn will conduct enrichment analyses on the metabolite panels and will provide much needed expertise that will enhance our ability to interpret the complex metabolite pathways that are involved.

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."

The initial findings on lipid metabolites have been accepted for presentation at the American College of Neuropsychopharmacology Annual Meeting on December 10, 2018:

> Inslicht S.S., Bhargava, A., Olshen A., Lujan, C., Neylan, T.C. The Lipidome in PTSD. The American College of Neuropsychopharmacology Annual Meeting, December 9-13, 2018, Hollywood, FL

A related manuscript on secondary variables from this dataset has been published:

> Inslicht, S.S., Rao, M.N., Richards, A., Gibson, C., Metzler, T.J., Neylan, T.C. Sleep and HPA Axis Responses to Metyrapone in Posttraumatic Stress Disorder. Psychoneuroendocrinology. 2017 Dec 7; 88:136-143. PMID: 29268182

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	**Sabra Inslicht**
Project Role:	Principal Investigator
Nearest person month worked:	1 person month
Contribution to Project:	Dr. Inslicht has expertise in psychophysiology and the neuroendocrinology of PTSD. Dr. Inslicht assumes overall scientific and administrative responsibility for this project, ensuring that research goals are met in a timely manner with scientific integrity. She has designed and is implementing each phase of the research plan. She is working with the study coordinator to oversee human subjects regulatory documentation and compliance, coordination of personnel involved in this protocol, the coordination of assay completion, as well as the development of a data tracking system to manage participant information, biological samples, and assay data. Over the next reporting period, Dr. Inslicht will work with the statistician to conduct data analyses and will prepare manuscripts and disseminate findings.

Name:	**Thomas Neylan**
Project Role:	Co-Investigator
Nearest person month worked:	*1 person month*
Contribution to Project:	Dr. Neylan has extensive expertise in the biology of PTSD, sleep, metabolic function, clinical trials, and laboratory-based psychophysiological research. He provides onsite support to Dr. Inslicht on the conduction of the proposed project, interpretation of sleep and HPA axis data, and will be involved in data analysis and manuscript preparation.

Name:	**Aditi Bhargava**
Project Role:	Co-Investigator
Nearest person month worked:	*1 person month*
Contribution to Project:	Dr. Bhargava is molecular biologist with extensive research experience in the area of neuroendocrinology, including pain, stress, and inflammation. Dr. Bhargava is responsible for design, execution, data analysis, and manuscript preparation. She is also responsible for conduction of assays in collaboration with colleagues at the UC Metabolomics Core.

Name:	**Callan Lujan**
Project Role:	*Study Coordinator/Staff Research Associate*
Nearest person month worked:	*6 person months*
Contribution to Project:	Ms. Lujan prepares all regulatory submissions to the IRB and VA Research and Development Committee and oversees compliance. Ms. Lujan supervises and coordinates study personnel, assists with sample organization and shipping, and the coordination of assay completion. Ms. Lujan has been working with the SFVA Stress and Health program data manager to develop a data tracking system to manage participant information, biological samples, and assay data.

Name:	**Olga Mayzel**
Project Role:	Database Manager

Nearest person month worked:	1 person month
Contribution to Project:	Ms. Mayzel has created a database for tracking biological samples, participant information, and metabolomics data collection. The database manager oversees database operations and will maintain all computer equipment including a main data server.

Name:	**Thomas Metzler**
Project Role:	Biostatistician
Nearest person month worked:	1 person month
Contribution to Project:	Mr. Metzler will continue to work closely with the investigators to complete correlate measure analyses.

Name:	**Ritu Roy**
Project Role:	Biostatistician
Nearest person month worked:	1 person month
Contribution to Project:	Dr. Roy has provided bioinformatics support for the metabolomics data and will continue to provide statistical support to complete metabolite analyses.

Name:	**Oliver Fiehn, PhD**
Project Role:	Consultant
Nearest person month worked:	1 person month
Contribution to Project:	Dr. Fiehn is the director of the West Coast Metabolomics Center at UC Davis. He is currently reviewing the outputs from the completed assays and will determine if additional assays are required.

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
Not applicable

QUAD CHARTS:
Quad chart attached as page 13

9. APPENDICES:

Nothing to report

Metabolomics: A Window for Understanding Long-Term Physical Consequences of Disturbed Sleep and Hypothalamic-Pituitary-Adrenal Function in Posttraumatic Stress
PR152209
W81XWH-16-1-0313

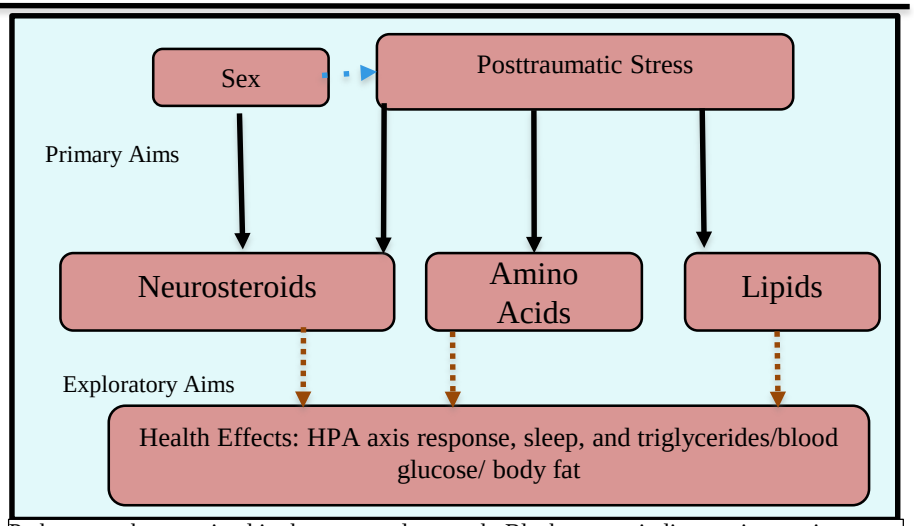
PI: Sabra Inslicht, PhD **Org: :** Northern California Institute for Research and Education (NCIRE) **Award Amount:** Direct $199,115 (Directs only)

Study/Product Aim(s)

1.Ascertain the neurosteroid (including glucocorticoid) metabolite profile in plasma of male and female patients with PTS, and in healthy controls.
2.Ascertain the primary amino acid and lipid metabolite profiles in plasma of male and female PTS patients, and in healthy controls.

Approach

Metabolomic analysis will be performed on pre-collected plasma samples from a study that had a two-group cross-sectional design in which main comparisons were with medically healthy medication-free male and pre-menopausal female subjects with chronic PTS (N= 44) and trauma-exposed, age-matched controls (N= 44). Previously collected measures, including sleep EEG and metabolic markers (e.g., fasting glucose, insulin response to oral glucose tolerance test (OGTT)), fasting lipids, and leptin will also be available for analysis.



Pathways to be examined in the proposed research. Black arrows indicate primary aims. Red arrows indicate exploratory aims. Blue arrows indicate previously established relationships.

Timeline and Cost

Activities CY	16	17
Study Start Up and Approvals		
Coordinate Staff for Sample Analysis		
Assay Biological Samples)		
Data Analysis and Grant Preparation		
Estimated Budget ($200K)	**$100k**	**$100k**

Updated: (September 28, 2018)

CY16 Goals – Study Setup and Assays
☑Hiring and Training of Study Staff
☑Coordinate with laboratory personnel for sample shipments
☑Build database for incoming data
☑Ship stored samples to the receiving laboratory and acquire data
CY17 Goal – Production readiness
☑Enter data and maintain database
❑ Address Aims 1& 2
❑ Work with Biostatistician to conduct analyses
❑ Prepare grant application for DOD or VA Merit Award funding for a clinical trial based on study findings

Comments/Challenges/Issues/Concerns

- Assays are still in progress, reflected in expenditures to date.

Budget Expenditure to Date
Projected Expenditure: $199,115 (Directs)
Actual Expenditure: $117,946 (Directs)