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TITLE: The Role of Sleep in Mediating Post-Traumatic Stress Disorder

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14. ABSTRACT Psychological and/or physical trauma can result in Post-traumatic stress disorder (PTSD). Commonly, PTSD presents with sleep disturbances. However, little is known about the contribution of sleep to the acquisition and maintenance of PTSD symptoms. The goal of this proposal is to first assess the contribution of sleep to the onset of PTSD. Using a rodent model of PTSD, we performed sleep deprivation experiments to determine if sleep loss prior to Single Prolonged Stress (SPS) trauma exposure is sufficient to exaggerate fear associated memory impairments. Experiments are ongoing, but preliminary data suggests that sleep loss prior to trauma does not enhance PTSD symptoms. Secondly, we assess the contribution of sleep to the maintenance of PTSD. For this, we are exposing animals to SPS followed by increasing sleep time via optogenetics. Experiments are ongoing, However, post-trauma-sleep restoration appears to be sufficient to alleviate fear associated memory impairments typically found following trauma exposure in rats.					
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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	1
2. Keywords	1
3. Accomplishments	1
4. Impact	4
5. Changes/Problems	5
6. Products	7
7. Participants & Other Collaborating Organizations	12
8. Special Reporting Requirements	12
9. Appendices	12

- 1. INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The current research is focused on understanding the contribution of sleep to the acquisition and maintenance of post-traumatic stress disorder (PTSD). The purpose of this research is to identify whether sleep or sleep disruptions contribute to the pathophysiology of PTSD.

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

Sleep, Post-Traumatic Stress Disorder, Sleep Deprivation, Stress

- 3. ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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: Perform Sleep deprivation/restriction and collect EEG/EMG recordings. To date, I have collected EEG/EMG recording and behavioral recordings from all but 8 animals. The final 8 Animals are ordered. The data collection portion of this Task is >90% complete. This aim is 2 months from completion.

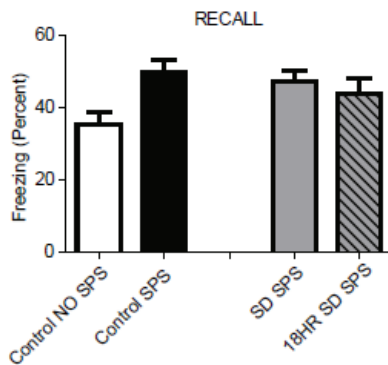
Major Task 2: Use Optogenetics to improve sleep after trauma exposure. I have recently begun the “quantification of PTSD severity and sleep phenotypes” as proposed in my SOW. I am 25% completed with this Task and am on target to complete the task by the 15 month mark proposed in the SOW.

Major Task 3: Preparation of Manuscripts. I am processing data and will begin manuscript preparation in the next 3-5 months.

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.

1) major activities; For Specific Aim1 I performed, 12 hr (acute) sleep deprivation experiments (n=9) and 18hr, 5day sleep restriction experiments (n=8). Sleep EEG and EMG data have been collected and are currently being processed to score behavioral state. Fear associated memory tests were performed on these, and control animals. As shown previously, (Vanderheyden) SPS results in impaired fear associated memory as measured by increased freezing on the previously mentioned fear recall test (Black bar compared to Control animals, White Bar). However, the addition of sleep deprivation (Grey Bar) or sleep restriction (Grey Bar with Stripes) did not further exaggerate the severity of the fear associated memory task.



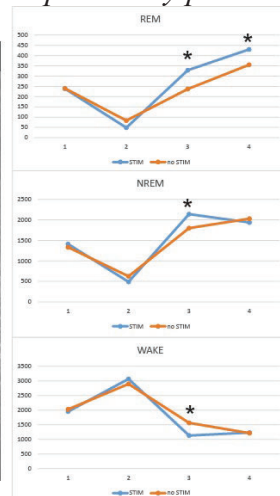
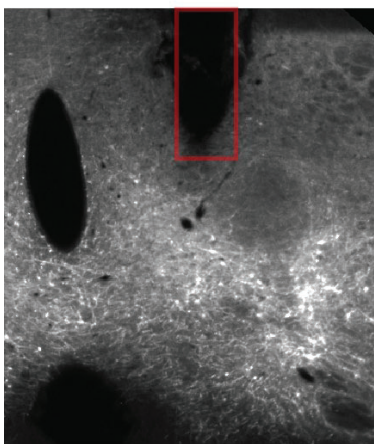
2) specific objectives; The objective of this aim was to assess whether sleep deprivation sensitized individual animals to trauma exposure. We hypothesized that sleep deprived animals would show greater impairment on this fear associated memory test.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); These data suggest that sleep deprivation prior to trauma exposure is insufficient to increase the severity of PTSD symptoms.

1) major activities; For Specific Aim2, I have completed 5 optogenetic stimulation experiments following trauma exposure. I am currently working to complete control animal testing for this portion of the aim along with control virus expression experiments.

2) specific objectives; For Specific Aim2 I proposed that sleep restoration via optogenetic enhancement of sleep time would rescue trauma associated fear memory impairments.

3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); I have found that the virus being used to express channelrhodopsin is expressed where it should be, and I am able to implant fiberoptic cables (track shown in red square) into the area where these cells are located (Bright white MCH containing cells; See figure below). In addition, preliminary evidence suggests that REM and NREM sleep is increased following opto stimulation (blue lines) compared to non-stimulation (orange lines) in agreement with previously published data using this construct.



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.

Nothing to report

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.

Nothing to report

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

If this is the final report, state “Nothing to Report.”

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

The experiments are on schedule and I will be finishing data collection in the next 2 months and finishing the analysis of the data thereafter.

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).

The findings on pre-trauma sleep not contributing to ptsd severity are very interesting and suggest that sleep before trauma is less important than sleep after trauma exposure and may, someday, determine how trauma exposed humans are treated for sleep interruptions immediately following a trauma.

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.

We are working to expand these behavioral findings and further our molecular and anatomical understanding of these phenotypes.

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.*

These findings may impact how trauma exposed individuals are treated in many ways. For example, car crash victims may be allowed to rest after a an accident instead of having their sleep disturbed while in the hospital. Additionally, individuals in the theatre of war may need to be removed to recover their sleep in a safe place.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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:*

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 Optogenetics portion of the proposed experiments required more troubleshooting than anticipated. For example, fabrication of the optical probes and verification of viral construct expression took longer than expected. However all issues have been resolved and data collection is proceeding on schedule.

Some unexpected outcomes also resulted in delays. Faulty dental acrylic resulted in lost EEG headpieces. Some behavioral data (freezing) due to improper use of new software.

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.

Although delays in data collection occurred, expenditures remained on track.

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

No human subjects were used in this research.

Significant changes in use of biohazards and/or select agents

none

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).*

In preparation

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).

None

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.*

None

- **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.

n/a

- **Technologies or techniques**
Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

n/a

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. 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.

n/a

- **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;*
- *physical 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.*

n/a

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”.

Example:

Name: Mary Smith

Project Role: Graduate Student

Researcher Identifier (e.g. ORCID ID): 1234567

Nearest person month worked: 5

Contribution to Project: Ms. Smith has performed work in the area of combined error-control and constrained coding.

Funding Support: The Ford Foundation (Complete only if the funding support is provided from other than this award.)

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 Principal Investigator (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.*

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.*