AWARD NUMBER: **W81XWH-16-1-0166**

TITLE: **Therapeutic Sleep for Traumatic Brain Injury**

PRINCIPAL INVESTIGATOR: Ravi **Allada**

CONTRACTING ORGANIZATION: **Northwestern University**
Evanston, IL 60208

REPORT DATE: **June 2018**

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE June 2018	2. REPORT TYPE Annual	3. DATES COVERED 1 Jun 2018 - 31 May 2018

4. TITLE AND SUBTITLE

Therapeutic Sleep for Traumatic Brain Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0166

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Ravi Allada

Bart van Alphen

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Northwestern University
Department of Neurobiology
2205 Tech Drive
60202
Evanston, IL

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This proposal will test the hypothesis that correcting sleep disorders can have a therapeutic effect on Traumatic Brain Injury (TBI) The majority of TBI patients develop sleep disorders, a correlation that is extremely prevalent in military personnel. Here, we have developed a paradigm to induce TBI in *Drosophila*. TBI induction results in increased mortality, impaired climbing behavior, decreased/fragmented sleep, cell death and altered gene expression in glia cells, including a strong innate immune response. We are currently testing whether sleep induction can restore these impairments, following promising pilot data showing that pharmacological sleep induction after TBI increases mortality while pharmacological wake induction decreases mortality.

15. SUBJECT TERMS

TBI, Traumatic Brain Injury, Sleep, Gene Expression, Drosophila

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	10	19b. TELEPHONE NUMBER *(include area code)*

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INTRODUCTION:

To study how TBI causes sleep disorders, and to study whether restoring normal sleep patterns, post-injury, can have a therapeutic effect, we need a model organism where baseline sleep can be recorded accurately, and where tools exist to induce either sleep or wake in such a way that TBI-induced sleep disorders can be corrected, by promoting wake in hypersomniacs and by promoting sleep in insomniacs. This approach is only feasible in *Drosophila*, as this model organism comes with a wealth of genetic tools that have been proven useful to modulate sleep and wake with high precision. We have developed a paradigm to reliably induce TBI in *Drosophila*.

ACCOMPLISHMENTS:

Major goals

We have completed most Major Tasks for Aim 1, as outlined in the Statement of Work.
Task 1A) Develop and test single fly TBI setup – **COMPLETED on 07/01/2016**
Task 1B) Determine TBI-induced sleep changes – **COMPLETED on 11/01/2016**
Task 1C) Determine TBI-induced behavioral changes – **COMPLETED on 01/01/2017**
Task 1D) Determine TBI-induced cell death – **COMPLETED on 08/01/2017**

Task 2A) Test the effect of sleep intervention on subsequent behavior and sleep architecture – **IN PROGRESS**
Task 2B) Test the effect of sleep intervention on subsequent markers of cell death – **ON HOLD** until Task 2A is completed

Keywords: TBI, Traumatic Brain Injury, Sleep, Gene Expression, Drosophila

What was accomplished under these goals?

Major Activity 1: to test the hypothesis that TBI causes either hypersomnia or insomnia in individual flies

Specific Objective 1A) Develop and test single fly TBI setup:
COMPLETED on 07/01/2016

Specific Objective 1B) Determine TBI-induced sleep changes:
COMPLETED on 11/01/2016

Specific Objective 1C) Determine TBI-induced behavioral changes:
COMPLETED on 01/01/2017

Other Achievements: To further explore the *Drosophila* immune response after TBI, we performed Translating Ribosome Affinity Purification and Sequencing (TRAP-Seq), which allows for a tissue or cell-type -specific manner of looking at the transcriptome of a given organism. This has previously been used to look at translated mRNAs in *Drosophila*. Here, we look at the glial transcriptome - glial activation is a biomarker for TBI. We performed TRAP-seq at 1, 3 and 7 days after TBI. TBI results in a large increase in genes that are up- or downregulated on day 1 (Fig 2A). On day 3 and 7 post-TBI, the number of upregulated genes is much lower. Contrary to our experimental design we noted that we also observed significant expression of neuronal genes suggesting that our purification is not complete. There is modest overlap between the genes that are up/down regulated on day 1 and days 3 or 7 (Fig 2B). Genes associated with the defense response are upregulated 24 hours after TBI, suggesting that TBI induces the activation of immune-related genes in glia after injury. Seven days after injury, genes associated with cell development, cell motion and cell morphogenesis are upregulated in one replicate, suggesting that glial development and proliferation occurs after injury (data not shown). Together, this biphasic response of increased expression in immune-related genes, followed seven days later by

increased expression in genes associated with cell development and morphogenesis serves as a validation of our head-specific TBI model.

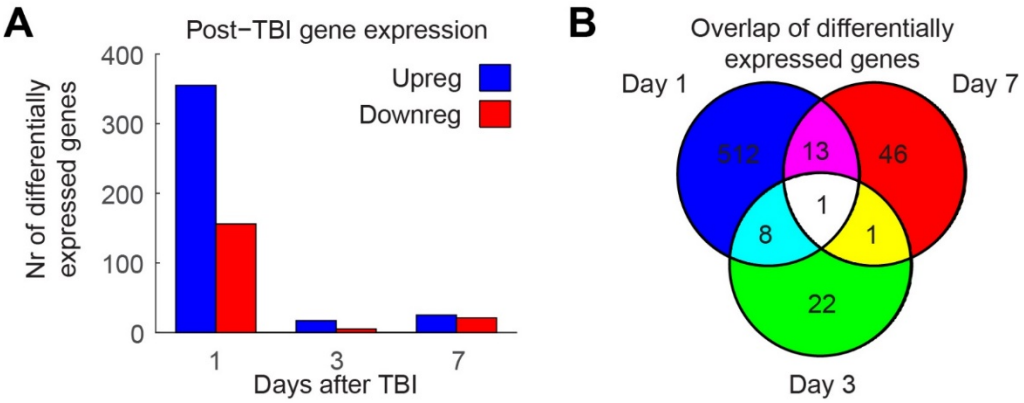

Figure 2 - TBI causes changes in glial gene expression

To test the role of the immune response in TBI survival and TBI-induced changes to behavior, we used the NFκB null mutant. This mutant lacks the master immune regulator NFκB Relish. This mutant is very sensitive to TBI induction, showing a mortality rate of 52% 24 hours after TBI (Fig 3A), much higher than the 15% 24 hour mortality rate showed in wild type flies (Fig 1A). Surprisingly, both climbing behavior and sleep are not affected by TBI in these flies (Fig 3B,C), suggesting that behavioral changes after TBI might be side effects of the immune response rather than the consequence of damage to the brain.

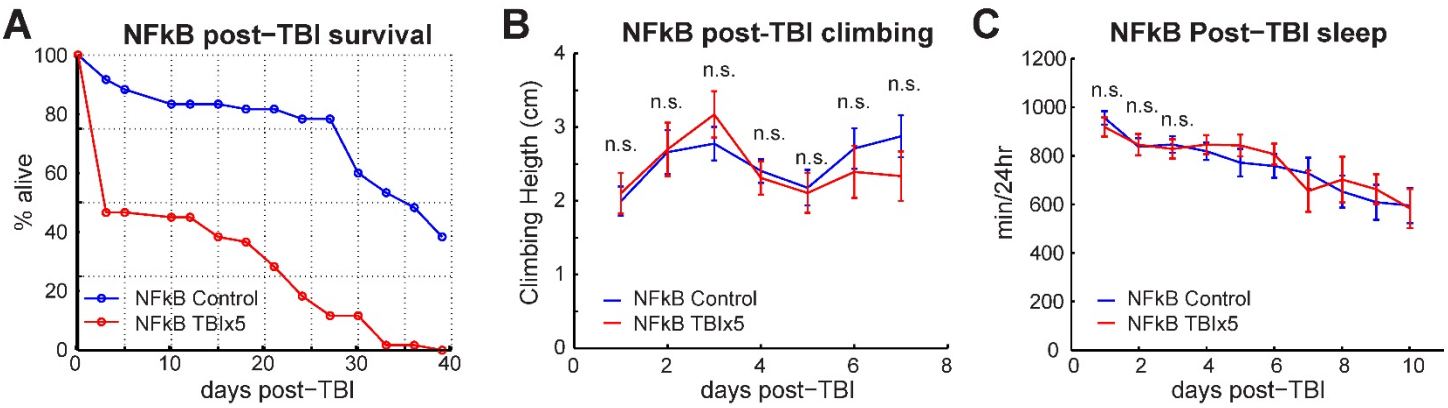

Figure 3 – TBI increases mortality in NFκB null mutants, without altering post-TBI behavior

Specific Objective 1D) Determine TBI-induced cell death

To test whether our TBI assay causes neuronal death, apoptosis was quantified using a TUNEL assay after inducing TBI by striking flies either 5 or 10 times and comparing the number of TUNEL positive cells at three different timepoints (4, 8 and 24 hours) between TBI-treated flies and sham-treated controls. Controls showed, on average, two to four TUNEL positive cells which may be spontaneous apoptotic cells (Fig 4A,B). Four hours after TBI induction we saw an increase in TUNEL positive cells in the TBIx10 condition at this time point. Eight hours after TBI induction we also saw an increase in TUNEL positive cells in the TBIx10 condition, but not in the TBIx5 condition. 24 hours after TBI induction we saw an increase in TUNEL positive cells in both the TBIx5 and the TBIx10 condition.

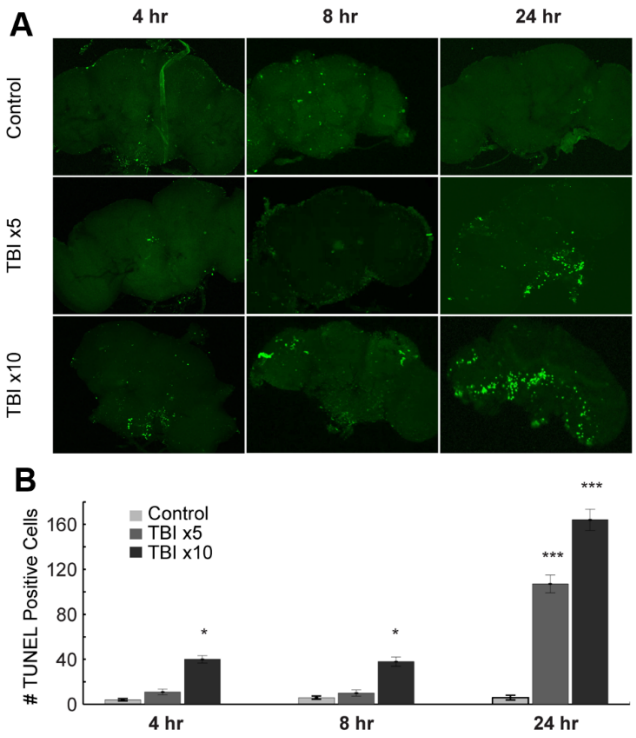

Figure 4– TBI induces cell death **A)** Representative images of TUNEL staining at different time points in control and post TBI flies that were hit either 5 (TBI x5) or 10 times (TBI x10). **B)** TBI increases TUNEL positive cells post TBI in a dose dependent manner.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
We have completed all goals for this Major Activity and are currently preparing this work for publication. It will also be presented at the Society for Neuroscience conference in San Diego in November this year.

Major Activity 2: to test the hypothesis that correcting impaired sleep patterns can facilitate post-TBI recovery

Specific Objective 2A) Test the effect of sleep intervention on subsequent behavior and sleep architecture – **IN PROGRESS**

We are currently testing how sleep and wake induction affects TBI-induced changes to mortality and behavior. We had difficulty replicating published sleep promoting GAL4 drivers, so we used a pharmacological approach to induce wake or sleep instead. We found that sleep is decreased and fragmented for the first three days after TBI induction (Fig 1C-F). To test whether correcting TBI-induced decreases in sleep would be beneficial to recovery and that further increasing wake would be detrimental, we fed flies food laced with either gaboxadol, a sleep promoting drug, or caffeine, a wake promoting drug. Both drugs were delivered at low concentrations that are sufficient to alter sleep without becoming toxic. Flies were fed this drug for three days after TBI induction, after which flies were transferred to normal food. Surprisingly, we found that increasing wake increases post-TBI survival while increasing sleep decreases post TBI survival (Figure 5). We are currently testing the effects of gaboxadol and caffeine on TBI-induced climbing impairments. We are also testing whether different sleep and wake promoting drugs have similar effects.

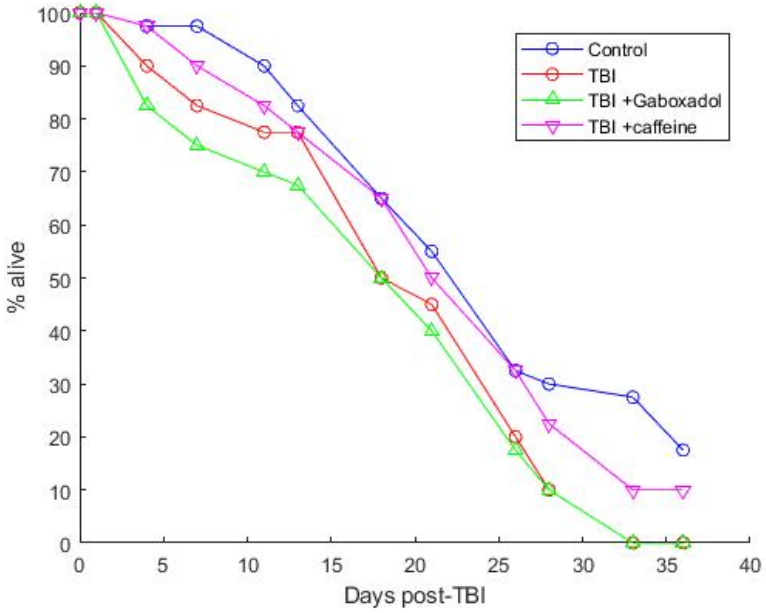

Figure 5 – Increasing sleep after TBI (through gaboxadol) increases mortality while decreasing sleep after TBI (through caffeine) decreases mortality.

Specific Objective 2B) Test the effect of sleep intervention on subsequent markers of cell death – **ON HOLD** until Task 2A is completed

In this part of the project, we will test whether sleep or wake induction after TBI will alter the amount and timing of cell death. This is still on hold until we have completed Objective 1D to determine when cell death occurs after TBI.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest?
Nothing to Report.

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

During the next reporting period, we aim to test the effect of sleep/wake induction on post-TBI lifespan, climbing behavior and cell death.

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

CHANGES/PROBLEMS

Nothing to Report

PRODUCTS

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*Bart van Alphen*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*?*
Nearest person month worked:	*7*
Contribution to Project:	*Dr van Alphen designed the project, developed the TBI paradigm, performed some sleep experiments and analyzed sleep data*
Funding Support:	-

Name:	*Anujaianthi Ramakrishnan*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*?*
Nearest person month worked:	*3.5*
Contribution to Project:	*Dr Ramakrishnan is performing the cell death assays*
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?

New Active Support

R01NS106955-01A1 05/01/18-02/28/23 1.5 academic, 2.5 summer
NIH/NINDS

Role: PI
Total R01: $1,728,125
Contact: Program Official Janet He, hey@ninds.nih.gov
Title R01: Molecular Mechanisms Integrating Circadian Timing and Photic Signaling
Project Goal: The goal of this proposal is to examine how circadian timing and photic signaling are integrated in the *Drosophila* clock neural network.
Specific Aims:
Aim 1. To identify the neuronal basis of PRL-1 effects on circadian and photoperiod-dependent diurnal behavior
Aim 2. To determine the relative roles of PRL-1 on the clock response to neural communication and on cell autonomous clocks
Aim 3. To test the hypothesis that PRL-1 functions in circadian and photoperiod-dependent diurnal behavior via TIMELESS

AARG-17-532626 03/01/18-02/28/21 0.25 summer
NIH/NINDS
Role: PI
Total: $150,000
Contact: Rita Freeman, Post Award Grant Specialist, rita.freeman@alz.org
Title*:* Discovery of Novel Mechanisms by which Sleep Modulates AB Toxicity
Project Goal: The goal of this proposal is to study the effect sleep deprivation on the toxicity of Alzheimer's related Abeta
Specific Aims:
Aim 1. To identify molecular pathways that mediate the effects of sleep deprivation on Aβ toxicity
Aim 2. To test the hypothesis that modifiers of sleep deprivation induced Aβ toxicity act via changes in Aβ levels, synapses, and/or cell death

2P01AG011412-18A1 09/15/17-05/31/22 0.45 academic, 0.15 summer
National Institute on Aging
Role: PI
Total: $22,308
Contact: Mack Mackiewicz, mackiewiczm2@mail.nih.gov
Title: Alterations of Sleep and Circadian Timing in Aging (Core C)
Project Goal: The proposal focuses on the interactions between peripheral tissue clocks, sleep and centrally regulated circadian rhythms in the age-related increase in metabolic disease.
Specific Aims:
Aim 1. To provide Projects 1 and 2 with reliable measurements and analyses of blood hormones appetitive, glucoregulatory, lipid, organic acid, and nucleotide blood constituents from human subjects who are affected by aging and/or sleep duration and quality.

Aim 2. To provide all projects with metabolic assays and gene expression analyses to investigate the role of the clock/NAD/sirtuin pathway in age-dependent decline in metabolic function and circadian behavior.
Aim 3. To provide genomic/transcriptome analyses using next generation sequencing (NGS) methods in animal models (Project 3)
Aim 4. To maintain and document assay quality and assure reliability of data produced from experiments required by the Projects.
Aim 5. To expand scope techniques as required for exploitation of new information and methods they become available.

What other organizations were involved as partners?

Nothing to Report

SPECIAL REPORTING REQUIREMENTS
Not applicable