

AWARD NUMBER: W81WH-20-1-0211

TITLE: Uncovering New Therapeutics and Neuroprotective Mechanisms for TBI

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CONTRACTING ORGANIZATION: Northwestern University, Evanston, IL

REPORT DATE: May 2022

TYPE OF REPORT: Annual

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

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REPORT DOCUMENTATION PAGEForm Approved
OMB No. 0704-0188

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1. REPORT DATE May 2022		2. REPORT TYPE Annual		3. DATES COVERED 01Apr2021-31Mar2022	
4. TITLE AND SUBTITLE Uncovering New Therapeutics and Neuroprotective Mechanisms for TBI				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81WH-20-1-0211	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Ravi Allada EEmail: r-allada@northwestern.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Northwestern University 633 Clark St. Evanston, IL 60208				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development 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 During the reporting period, we find that TBI induced changes in mortality, locomotion behavior and sleep. Screening individual candidate genes differentially expressed in the glial cells with RNAi, we investigate the effect of inhibition of gene expression in mortality, climbing behavior and sleep. We currently identify that knockdown of several candidate genes suppresses high mortality, reduced locomotion and impaired sleep. We find that phosphomimetic TauE14 expression driven by dopamine receptor promoter fragments in the small ventral lateral clock neurons decreases rhythmic power of locomotor activity and consequently induces circadian disruption. Importantly, these studies reveal molecular effectors of TBI effects highlighting the role of innate immunity and inflammation.					
15. SUBJECT TERMS Drosophila, sleep, innate immunity, traumatic brain injury					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 12	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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1. **INTRODUCTION:** Sleep disorders are highly associated with traumatic brain injury and may contribute to adverse outcomes. Here we exploit a novel *Drosophila* TBI model to reveal the underlying molecular mechanisms linking TBI to behavioral impairments including sleep.
2. **KEYWORDS:** *Drosophila*, sleep, innate immunity, traumatic brain injury
3. **ACCOMPLISHMENTS:**
 - **What were the major goals of the project?**
 - **Aim 1: To understand the cellular and molecular bases of the neuroprotective effect of NFkB**
 - **Aim 2: to uncover genes and pathways that are neuroprotective for TBI**
 - **Aim 3: to test FDA approved drugs for improving TBI-induced mortality and sleep disorders**
 - **What was accomplished under these goals?**
 - We discovered potentially important candidate genes in mediating TBI effects on sleep, mortality and climbing (Fig. 3 & 4)
 - We revealed specific locomotor impairments after TBI (Fig. 1 & 4)
 - We discovered a role for well-known effector of TBI effects, phosphorylated Tau on circadian behavior and axonal transport (Fig. 5-9).
 - **What opportunities for training and professional development has the project provided?**
 - During the reporting period, 2 postdoctoral fellows and 1 PhD student received training in *Drosophila* genetics, circadian rhythms and sleep analysis, and scientific presentation of results.
 - **How were the results disseminated to communities of interest?**
 - We plan to publish the results for the scientific community
 - **What do you plan to do during the next reporting period to accomplish the goals?**
 - We will perform a large-scale genetic screen to discover in vivo genetic mediators of TBI and/or phospho-Tau effects potentially discovering novel therapeutic targets.

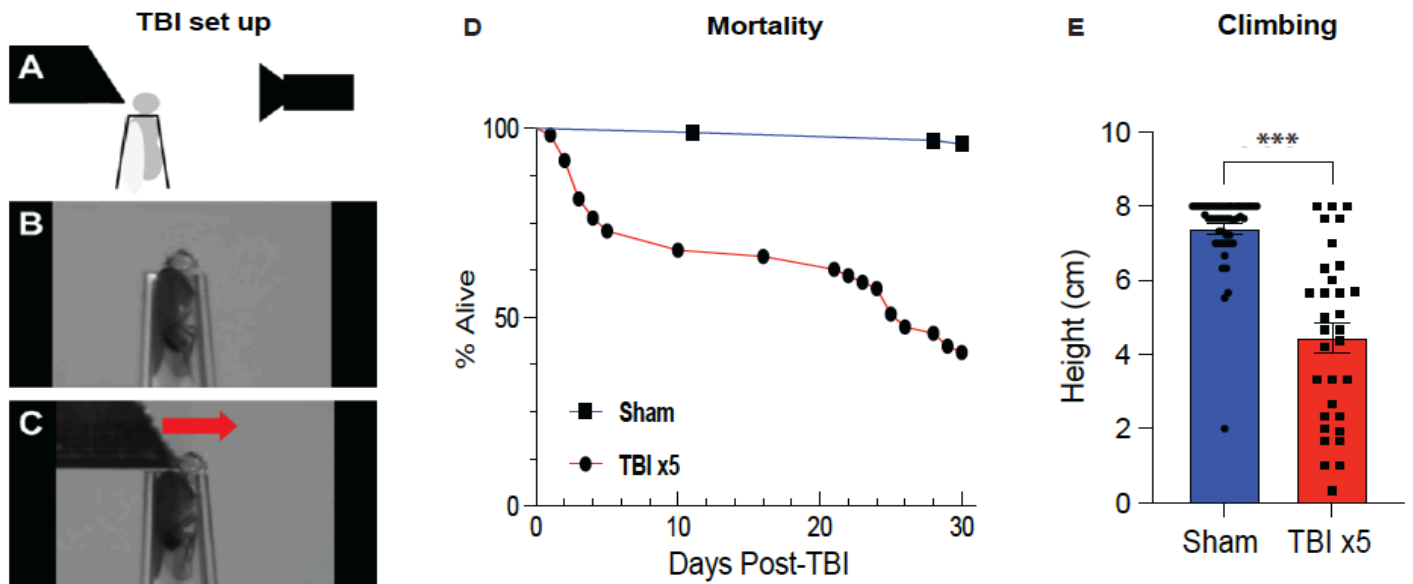


Fig. 1. TBI impacts mortality and climbing behavior. A-C) We set up *Drosophila* closed head TBI system with the pin of a pull-type solenoid, which delivered 8.34 Newtons of force. Using an isogenic strain (ISO31) single males got stuck at the end of the P200 pipette. Flies were observed using a high-speed camera to ensure that they were in the proper position. Five strikes were delivered to individual flies with 1-min interval between strikes. D) We measured survival after delivering TBI. 50% mortality was obtained approximately 25 days after TBI induction. E) TBI also impacts locomotion activity such as climbing behavior. We measured height single flies climbed for 4 sec and averaged distance of three trials with one-min interval between trials. *** $p < 0.001$.

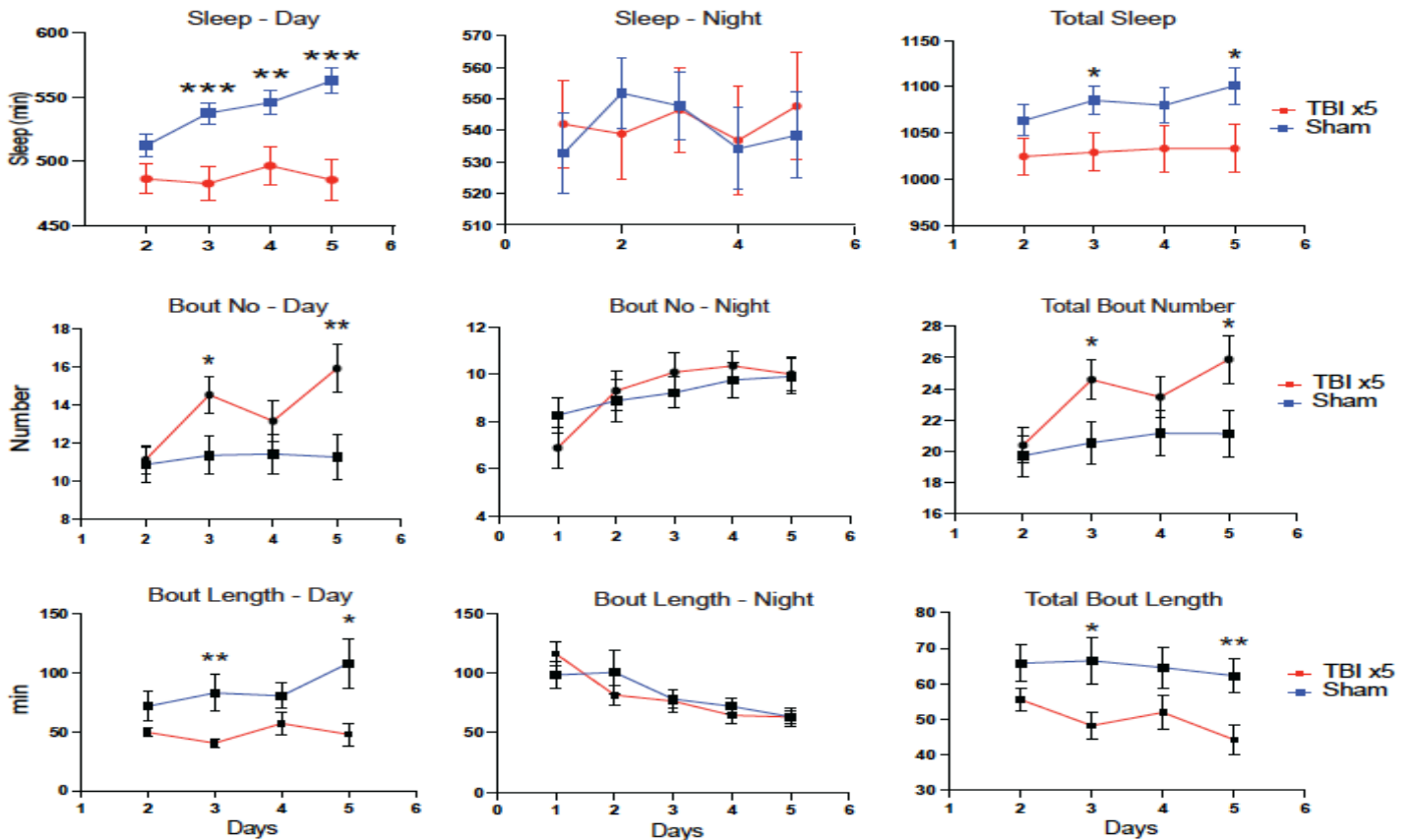


Fig. 2. Sleep is reduced in TBI survivors. Focusing on TBI survivors we closely examined sleep levels using the *Drosophila* Activity Monitoring (DAM) system in which individual flies were loaded into 65mm glass tubes with food and monitored at 25C incubator. We measured sleep amount, bout number and bout length during 12h-daytime and 12h-nighttime in 5LD: 7DD cycle. Daytime sleep was significantly disrupted in TBI survivors. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

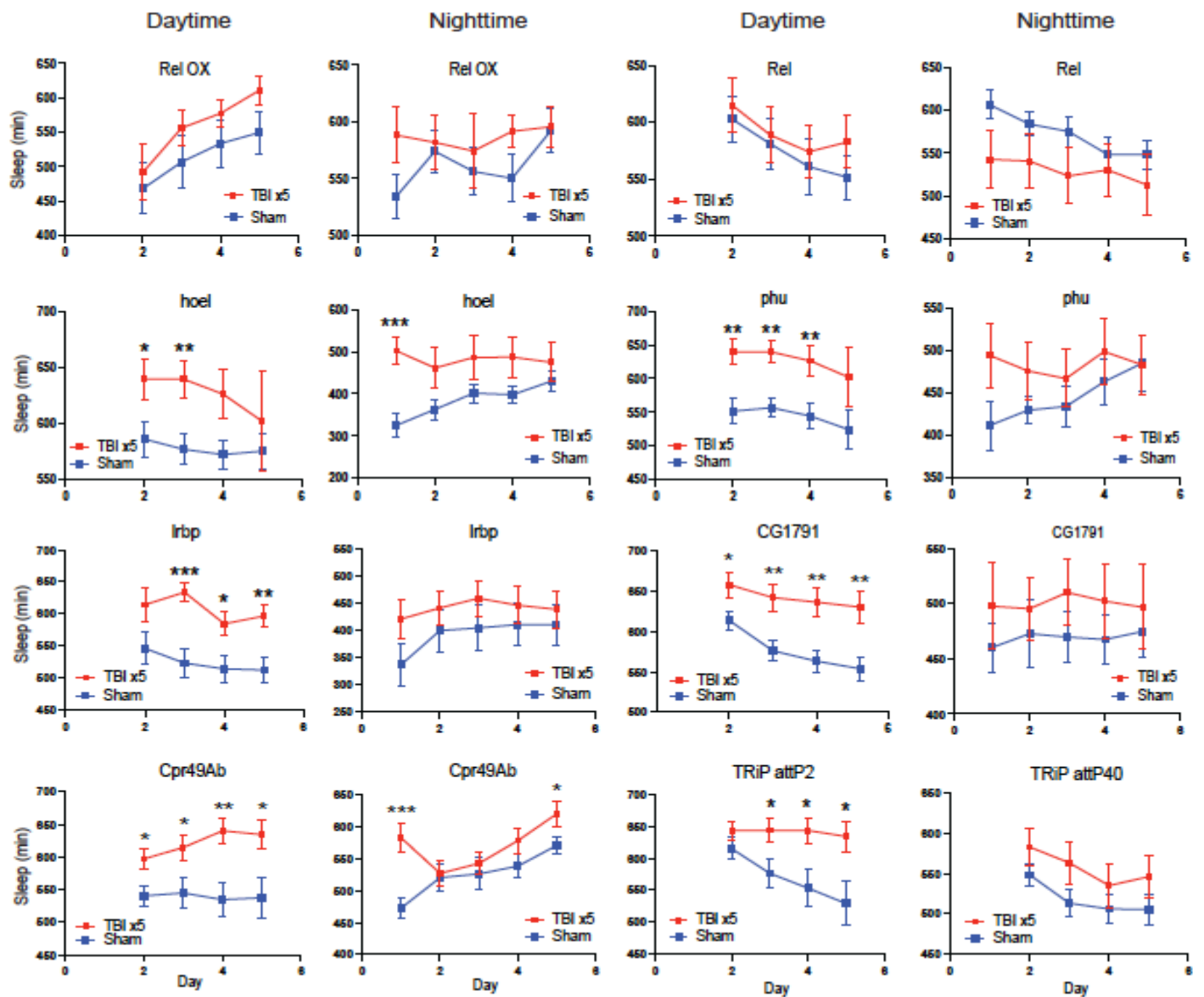


Fig. 3. Knockdown of some candidate genes increased TBI survivor sleep which was impaired by TBI. We identified ~350 genes differentially expressed in the glial cells using immunoprecipitation and TRAP-seq techniques. We inhibited expression of these genes with RNA interference methods and observed the inhibition effect on sleep during 12h daytime and 12h nighttime per day in the DAM system. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

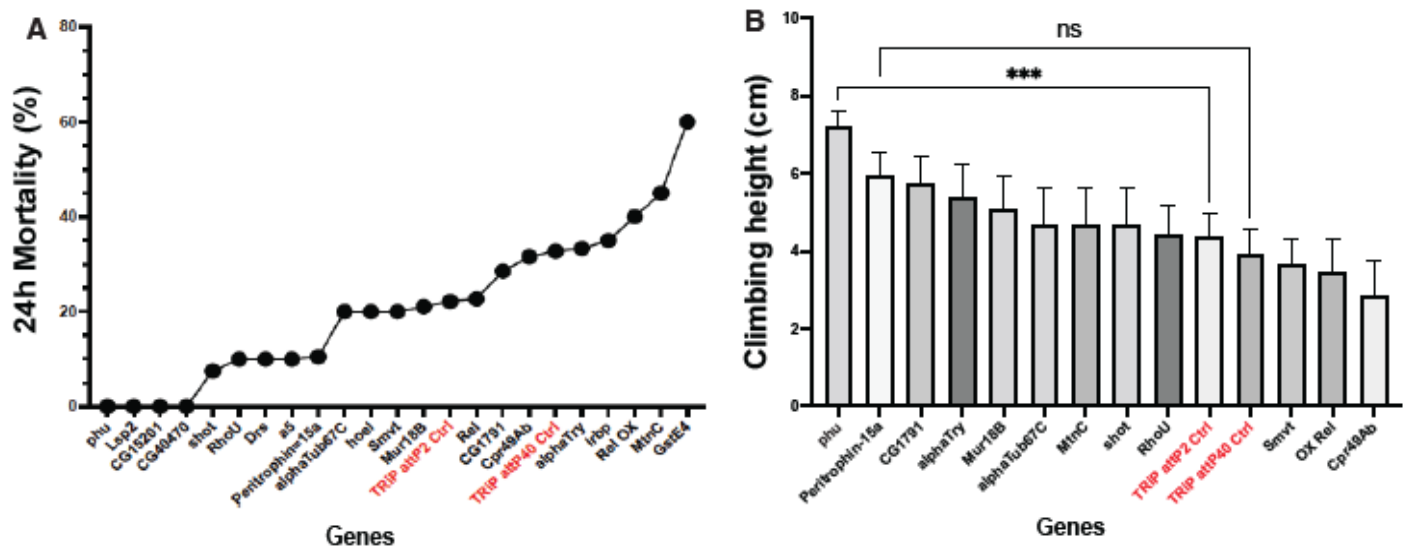


Fig. 4. Knockdown of candidate genes with RNAi lines significantly suppresses TBI-induced mortality and rescues reduced climbing activity. We measured percentages of dead flies (A), climbing distance (B) 24h after TBI induction and compared to its genetic controls, TRiP attP2 or TRiP attP40. For instance, knockdown of *phu* significantly improved reduced survival and climbing behavior as well as sleep behavior (Fig. 3). *** $p < 0.001$.

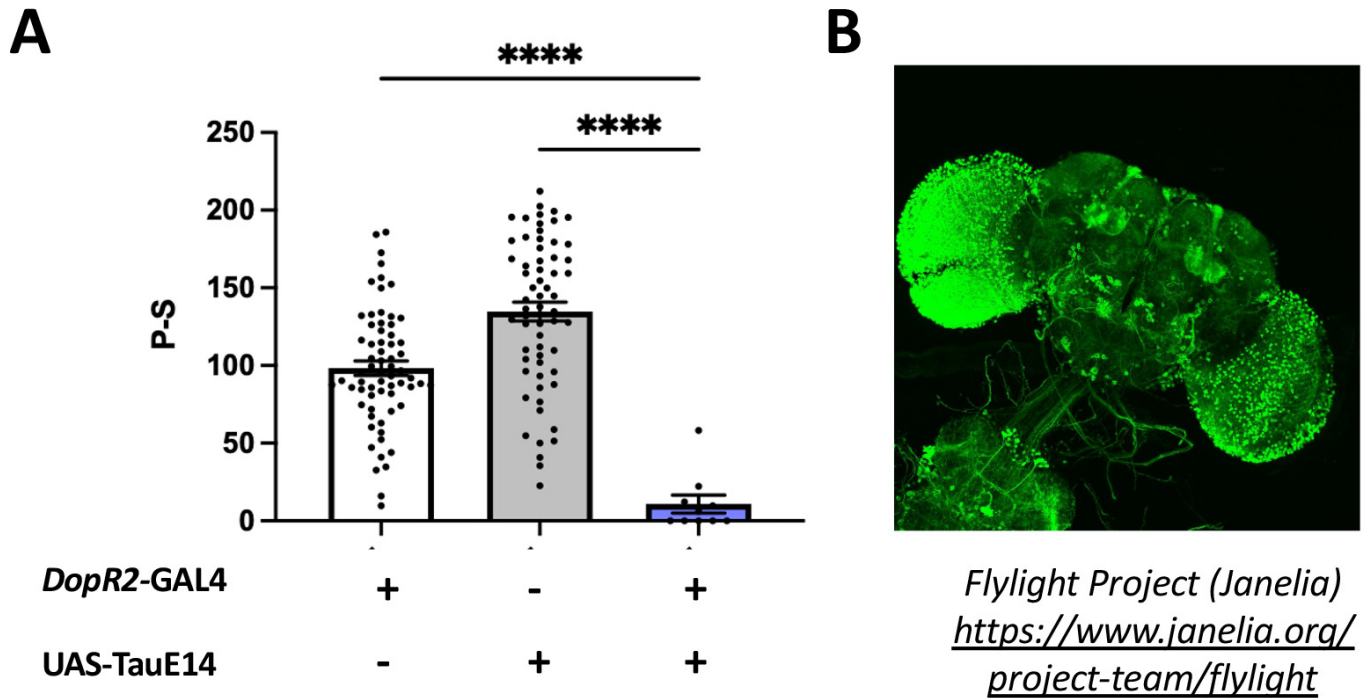


Fig. 5. Phosphomimetic TauE14 expression driven by a dopamine receptor promoter fragment decreases rhythmicity.

A. Rhythmic power of locomotor behavior (P-S) over 7 days in constant darkness (DD) for flies expressing UAS-TauE14(lavender) using the *DopR2-GAL4* driver (R70H10) compare to the UAS only (gray) and GAL4 only (white) controls. Statistical significance determined using one-way ANOVA followed by Bonferroni comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0005$, ns = not significant. Error bars shown in SEM. B. *DopR2-GAL4* (R70H10) expression from Flylight Project as visualized using a UAS-GFP reporter.

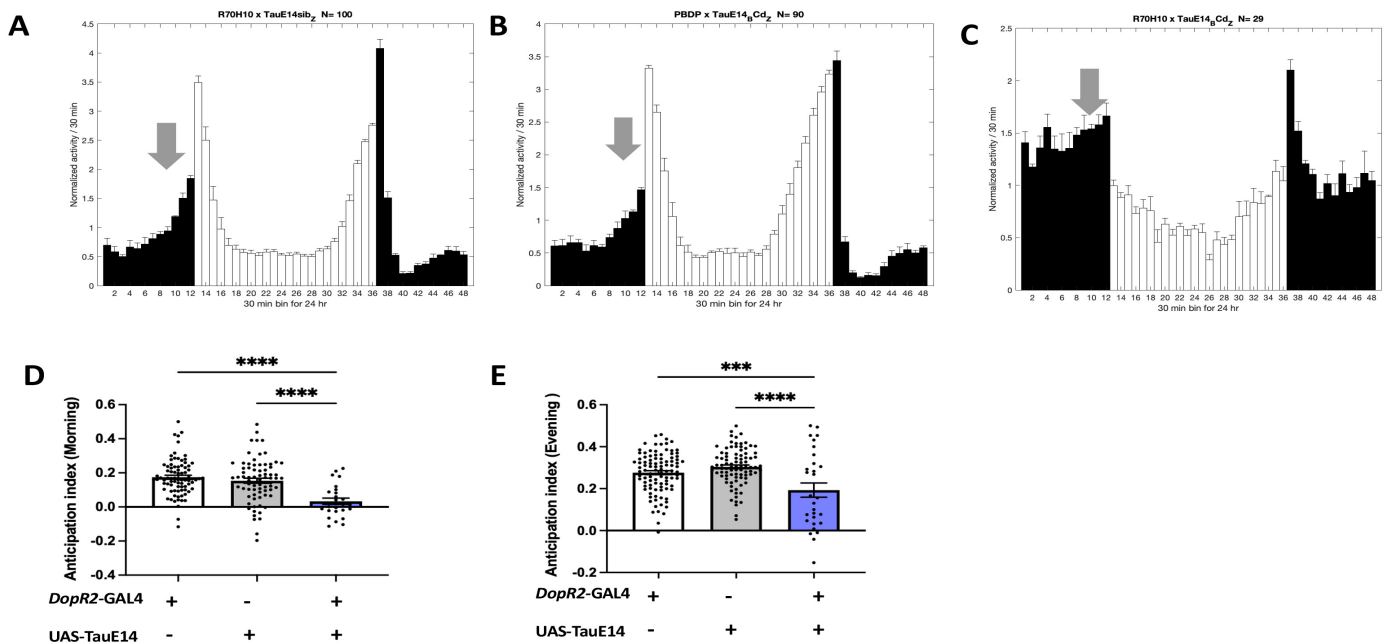


Fig. 6. Expression of phosphomimetic TauE14 in DopR2 neurons reduces morning and evening anticipation (A-C). Normalized activity profiles during 4 days of 12hour light: 12 hour dark conditions (LD) for (A) GAL4 only control, (B) UAS only control, or (C) flies expressing UAS-TauE14 with DopR2-GAL4 (R70H10). D. LD morning anticipation index for flies expression UAS-TauE14 with R70H10-GAL4 (lavender) driver compared to UAS only (gray) and GAL4 only (white) controls. E. LD evening anticipation index for flies expression UAS-TauE14 with R70H10-GAL4 (lavender) driver compared to UAS only (gray) and GAL4 only (white) controls. Statistical significance determined using one-way ANOVA followed by Bonferroni comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0005$, ns = not significant. Error bars shown in SEM.

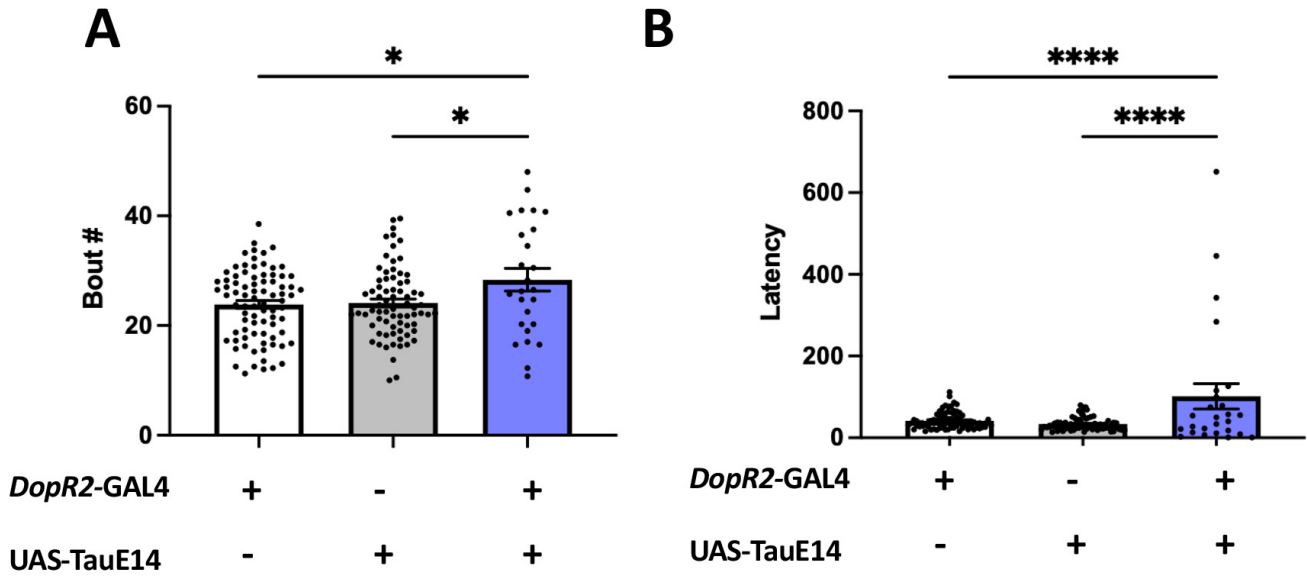


Fig. 7. Expression of phosphomimetic TauE14 in DopR2 neurons increases bout number and latency. Bout number (A) and latency (B) over 4 days of LD conditions for flies expressing UAS-TauE14(lavender) using the DopR2-GAL4 driver (R70H10) compare to the UAS only (gray) and GAL4 only (white) controls. Statistical significance determined using one-way ANOVA followed by Bonferroni comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0005$, ns = not significant. Error bars shown in SEM.

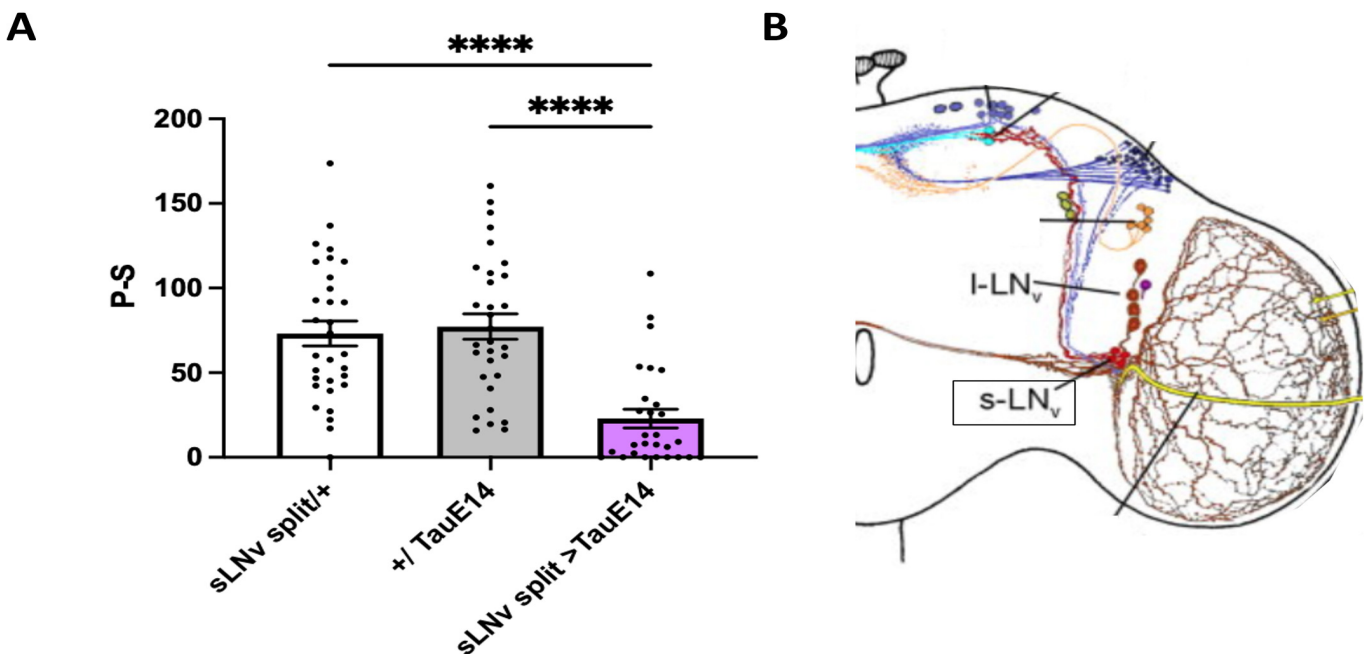


Fig. 8. TauE14 expression in the small ventral lateral clock neurons (s-LNv) decreases rhythmicity.

A. Rhythmic power of locomotor behavior (P-S) over 7 days in constant darkness (DD) for flies expressing UAS-TauE14 (purple) using the s-LNv driver compare to the UAS only (gray) and GAL4 only (white) controls. Statistical significance determined using one-way ANOVA followed by Bonferroni comparison test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0005$, ns = not significant. Error bars shown in SEM. B. Depiction of small ventral lateral neurons (s-LNv) location in the Drosophila brain (Helfrich Forster, 2006)

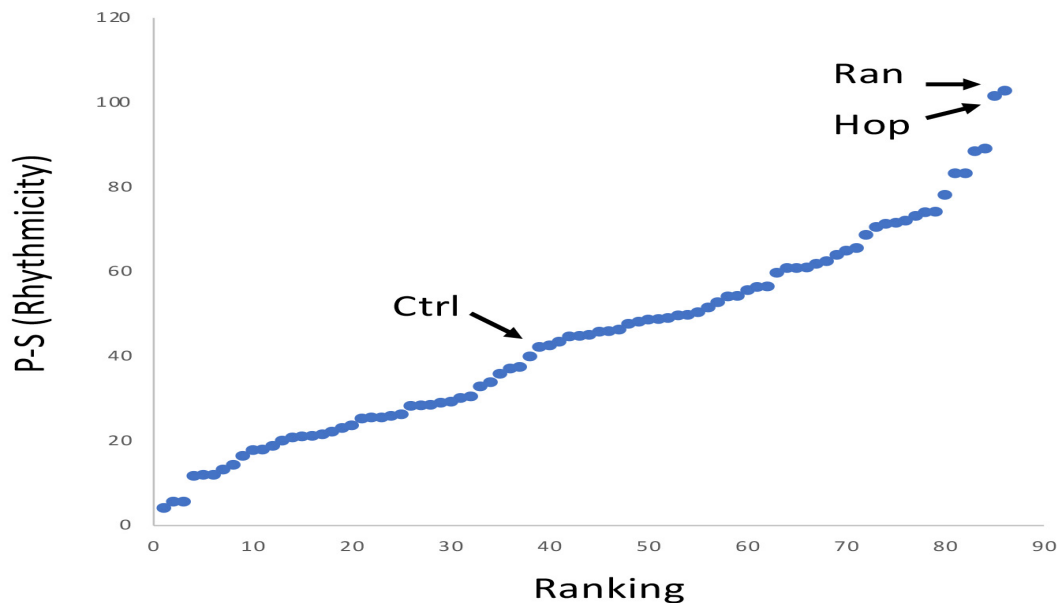


Figure 9. Genetic screen to identify suppressors of TauE14-induced behavioral phenotypes. Ranking of screened RNAi lines based on their average rhythmicity (power-significance [P-S]) values in pdfGAL4 UAS TauE14 flies. Ctrl indicates the average P-S of the control (pdfGAL4 UAS TauE14 X attP2). In the preliminary screen, RNAi knockdown of heat-shock protein organizing protein (Hop) or the Ran GTPase (arrows) substantially increased rhythmicity in pdfGAL4 UAS TauE14 flies.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
 - The discovery of the AMPs in mediating TBI effects provides important in vivo evidence for this pathway.
- **What was the impact on other disciplines?**
 - These studies may also highlight novel treatments for TBI
- **What was the impact on technology transfer?**
 - N/A
- **What was the impact on society beyond science and technology?**
 - If successful, new treatments for TBI could improve the lives of affected individuals.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
 - Beyond being slowed by the COVID-19 pandemic and an untimely death, we did not change our overall approach.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - The COVID-19 pandemic led to a lab shutdown and thus significantly slowed our research progress. As the pandemic is resolving, we have restarted our experiments.
 - One of the postdoctoral fellows who was leading our TBI studies unexpectedly passed away, temporarily stopping his experiments. We have trained new personnel and are now reinitiating our plans. We are moving to a technically simpler injury assay to facilitate our studies.

- **Changes that had a significant impact on expenditures**
 - Nothing to report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
 - Not applicable

6. PRODUCTS:

- **Publications, conference papers, and presentations**
 - **Journal publications.**

van Alphen B, Stewart S, Iwanaszko M, Xu F, Li K, et al. (2021). Glial immune-related pathways mediate effects of closed head traumatic brain injury on behavior and lethality in *Drosophila*. *PLoS Biol* 20(1): e3001456.
<https://doi.org/10.1371/journal.pbio.3001456>

Zhang MY, Lear BC, Allida R. (2022). The microtubule-associated protein Tau suppresses the axonal distribution of PDF neuropeptide and mitochondria in circadian clock neurons. *Hum Mol Genet* 31(7), 1141-1150. DOI: [10.1093/hmg/ddab303](https://doi.org/10.1093/hmg/ddab303)

- **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

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

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

Name:	<i>Eugene Nyamugenda</i>
Project Role:	<i>Postdoctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>6</i>
Contribution to Project:	<i>Dr. Nyamugenda has developed novel strategies to study TBI related Tau</i>
Funding Support:	<i>This award</i>

Name:	<i>Yong-Kyu Kim</i>
Project Role:	<i>Senior Research Associate</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>6</i>
Contribution to Project:	<i>Dr. Kim analyzed behavioral and genetic effects of TBI and performing training</i>
Funding Support:	<i>This award</i>

Name:	<i>Ravi Allada</i>
Project Role:	<i>Principal Investigator</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>He supervised this project</i>
Funding Support:	<i>This award</i>

Name:	<i>Melanie Zhang</i>
Project Role:	<i>PhD Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>4</i>
Contribution to Project:	<i>She characterized the effects of TBI related phospho-Tau</i>
Funding Support:	<i>This award</i>

Name:	<i>Clark Rosensweig</i>
Project Role:	<i>Postdoctoral Fellow</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Dr. Rosensweig is responsible for establishing sleep assays relevant to TBI</i>
Funding Support:	<i>This award</i>

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

2022 Award (PI: Allada), Les Turner ALS Center, Analysis of Ataxin2 Targets as Mediators of Amyotrophic Lateral Sclerosis (pending to active)

W81XWH-22-1-0217, (PI: Allada), Department of Defense, Discovery of In Vivo Molecular Pathways Mediating Tau-Induced Sleep and Circadian Disruption (pending to active)

- **What other organizations were involved as partners?**
 - Lake Forest College

8. SPECIAL REPORTING REQUIREMENTS

- Not applicable

9. APPENDICES

- Not applicable