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TITLE: Uncovering New Therapeutics and Neuroprotective Mechanisms for TBI

PRINCIPAL INVESTIGATOR: Ravi Allada

CONTRACTING ORGANIZATION: Northwestern University

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

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

During the reporting period, we find that TBI induced changes in sleep are not related to TBI induced mortality with similar changes observed between flies that die and do not. We used high speed video analysis to resolve at high resolution the locomotor impairments induced in a dose-dependent manner by TBI. We discovered a role for antimicrobial peptides (AMPs) in mediating TBI effects on sleep where loss of AMPs alters the TBI induced effects on sleep (increased versus reduced in control flies). 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	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	11	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

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 a key and specific role for antimicrobial peptides in mediating TBI effects on sleep but not climbing ability (Fig. 5)
        - We revealed specific locomotor impairments after TBI in a dose-dependent manner (Fig. 1)
        - We demonstrated that TBI induced sleep effects are not related to mortality effects (Fig. 2-4)
        - We discovered a role for well-known effector of TBI effects, phosphorylated Tau on circadian behavior and axonal transport (Fig. 6, 7).

    - **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.

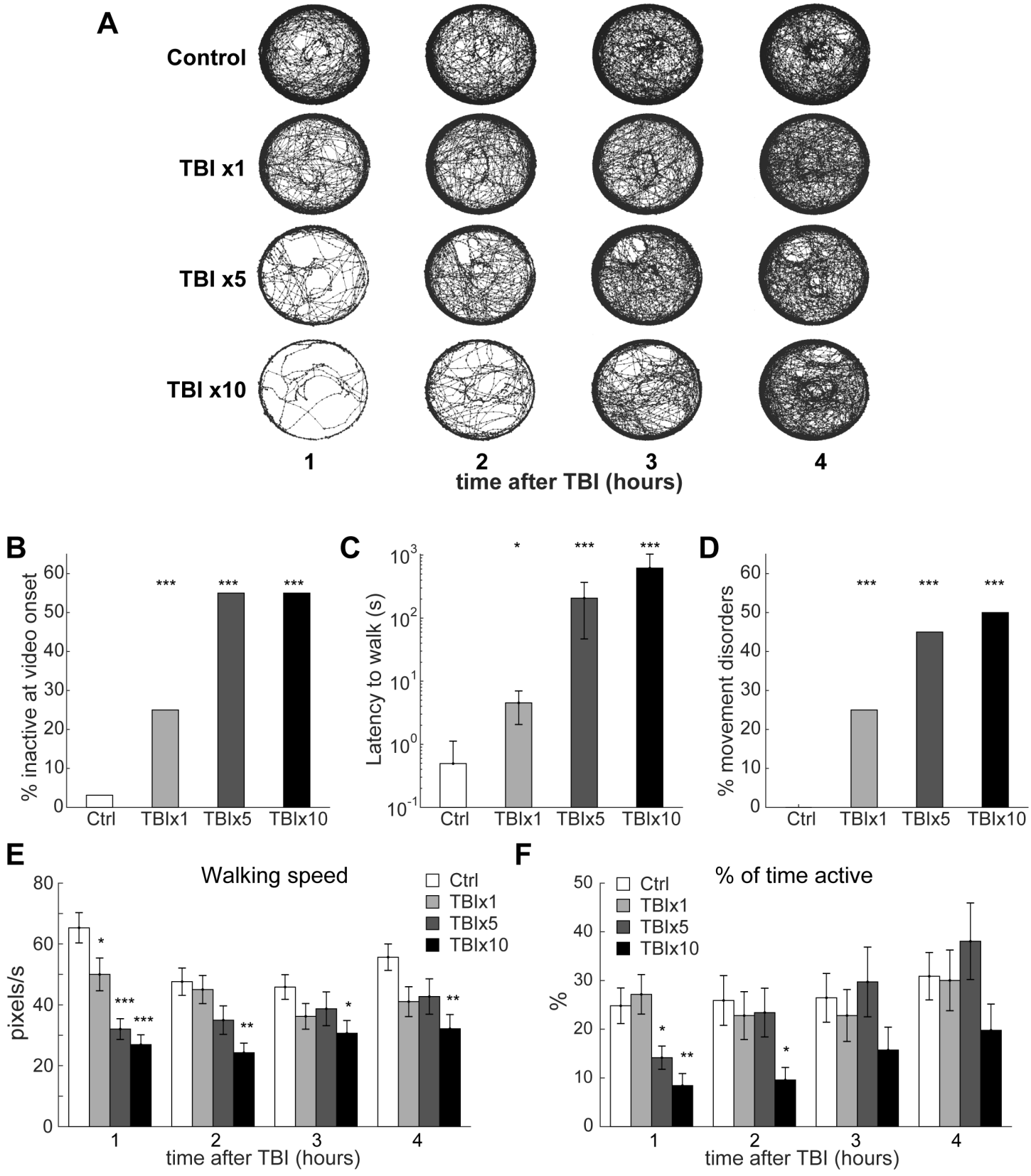

Fig 1. TBI results in immediate locomotion defects. Locomotion is measured using a high speed camera system.

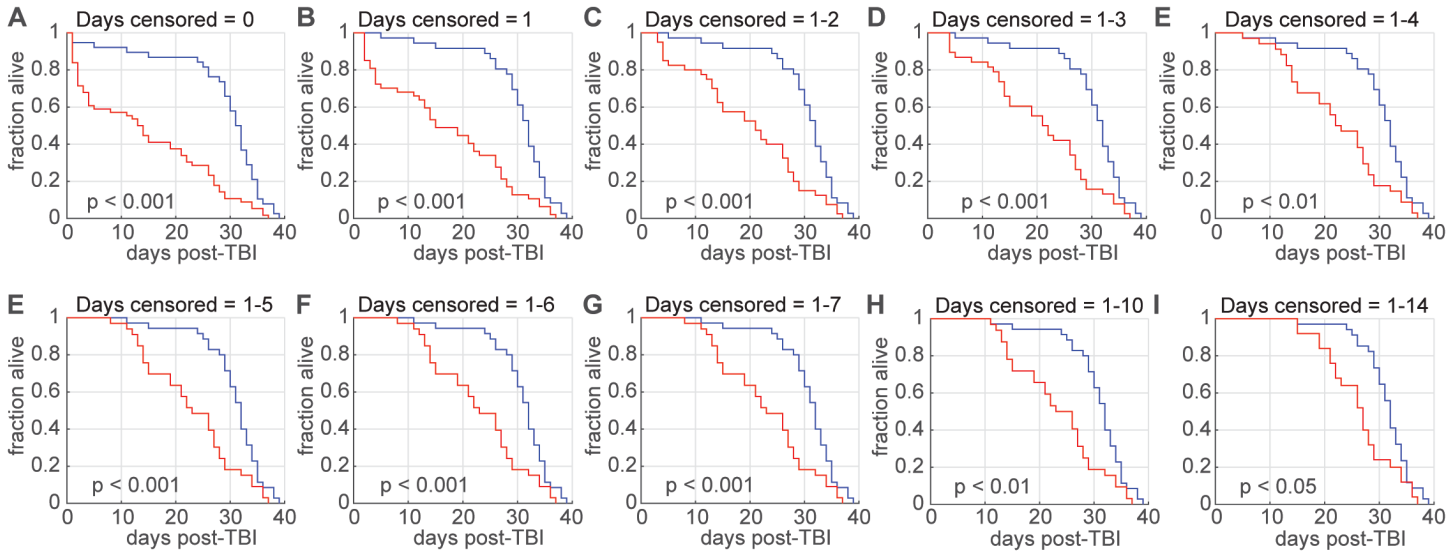

Fig. 2 TBI impacts mortality independent of early deaths. We removed early deaths and measured mortality after delivering TBI.

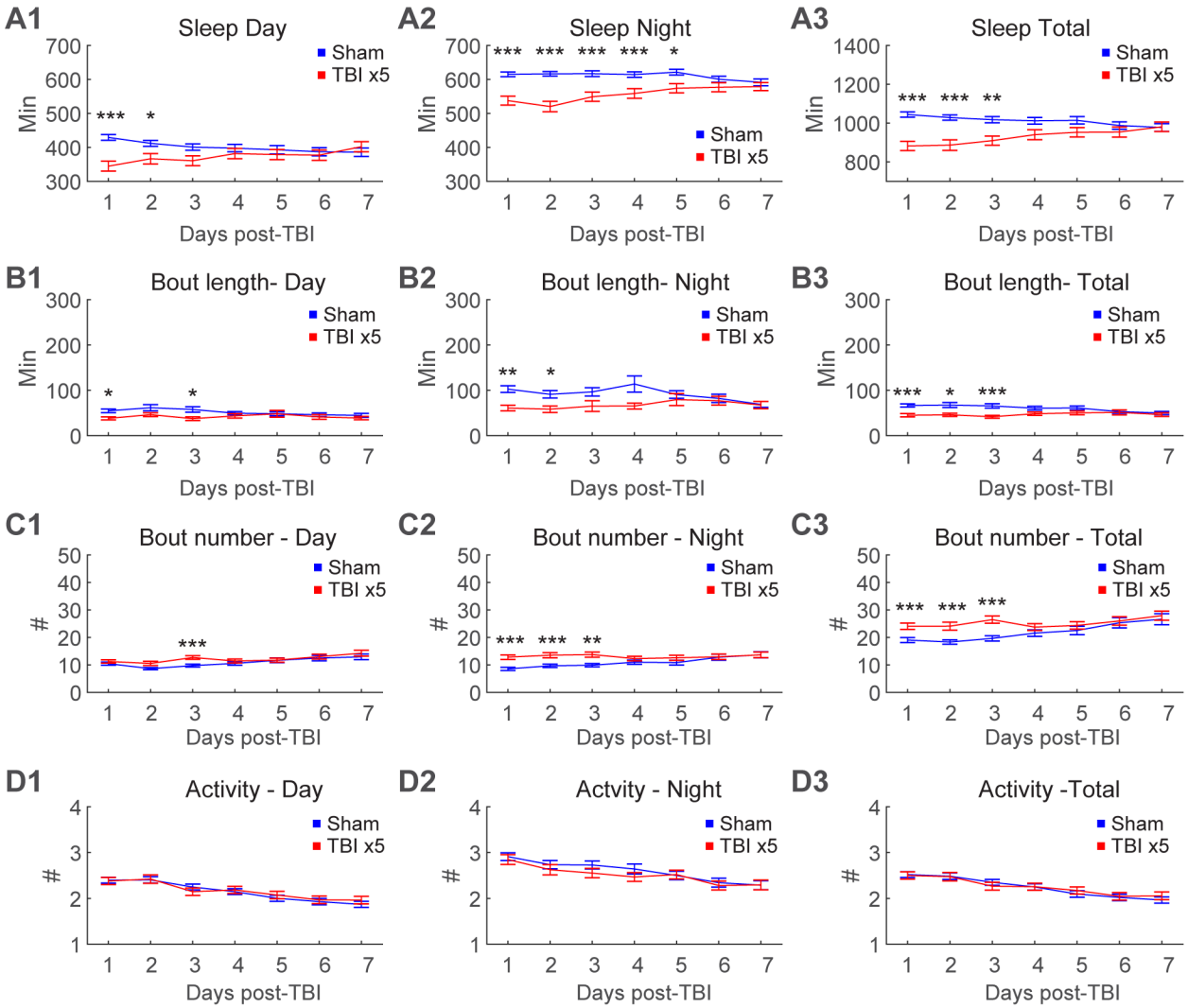

Fig. 3. Sleep is reduced in TBI survivors. Focusing on TBI survivors we closely examined sleep levels using the Drosophila Activity Monitoring system.

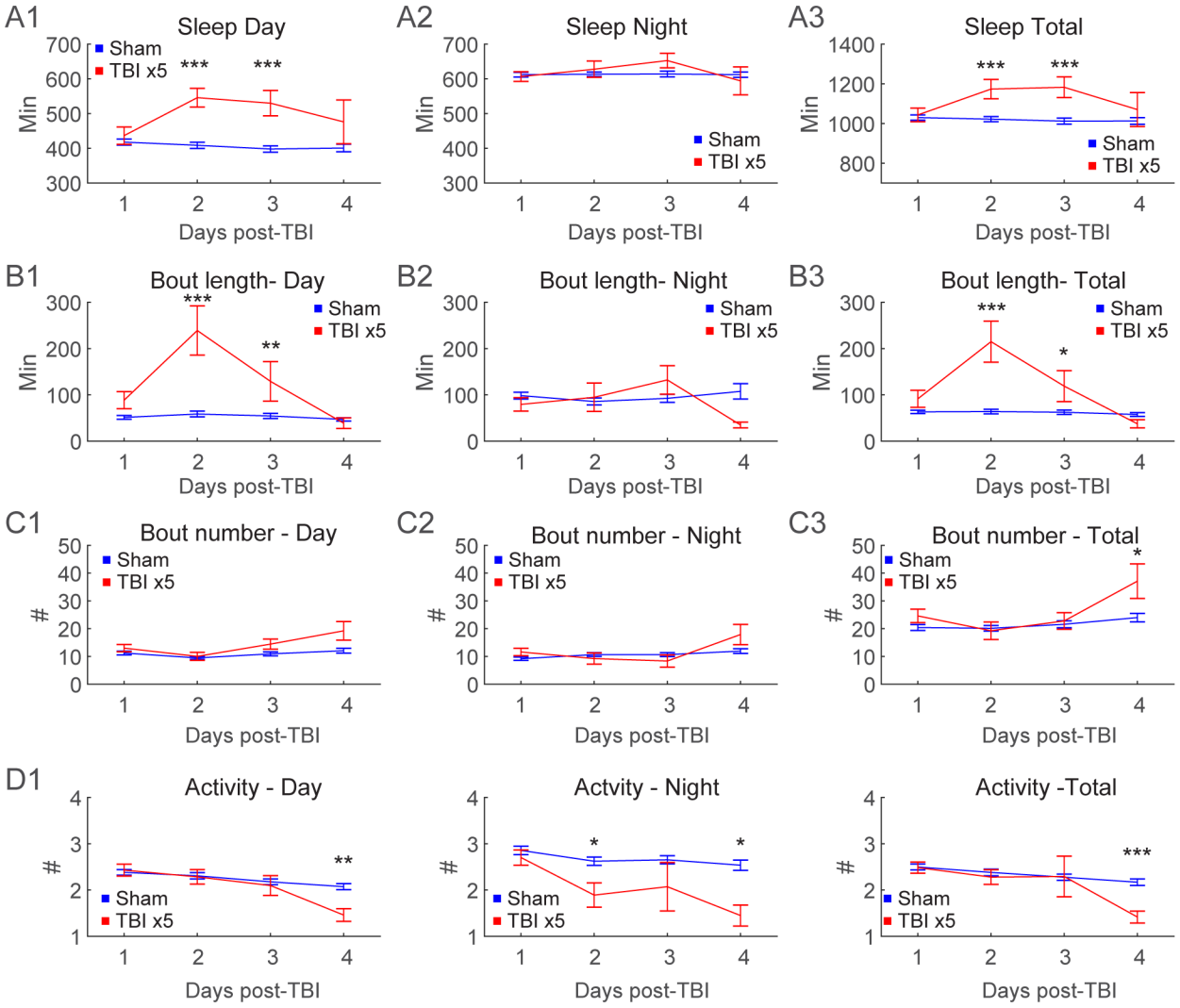

Fig. 4. Sleep is increased in dying flies. We also look at those flies that die and noted that sleep is increased.

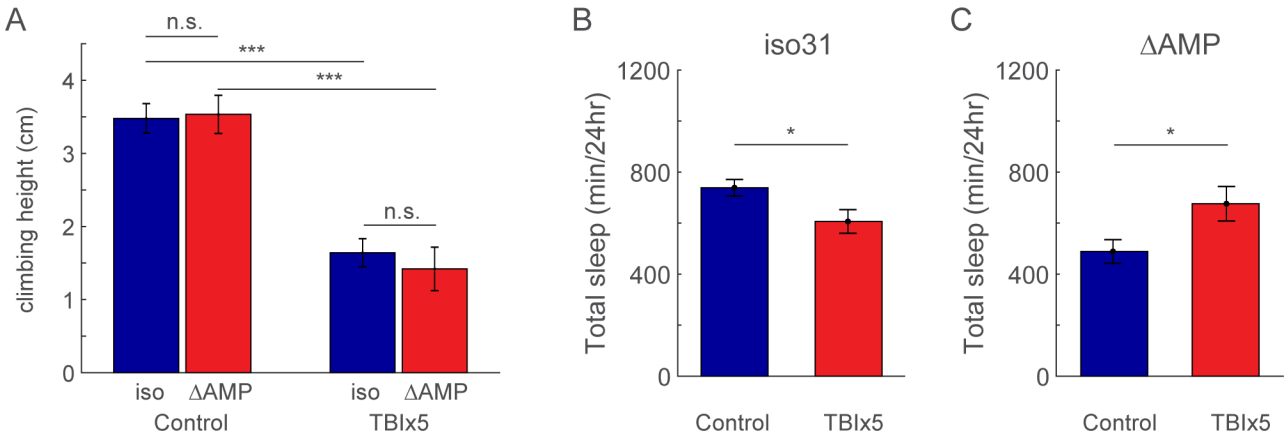

Fig. 5. Loss of antimicrobial peptides impacts TBI effects on sleep but not climbing ability. Deletion of several antimicrobial peptide genes did not impact climbing height achieved but did alter the sleep response.

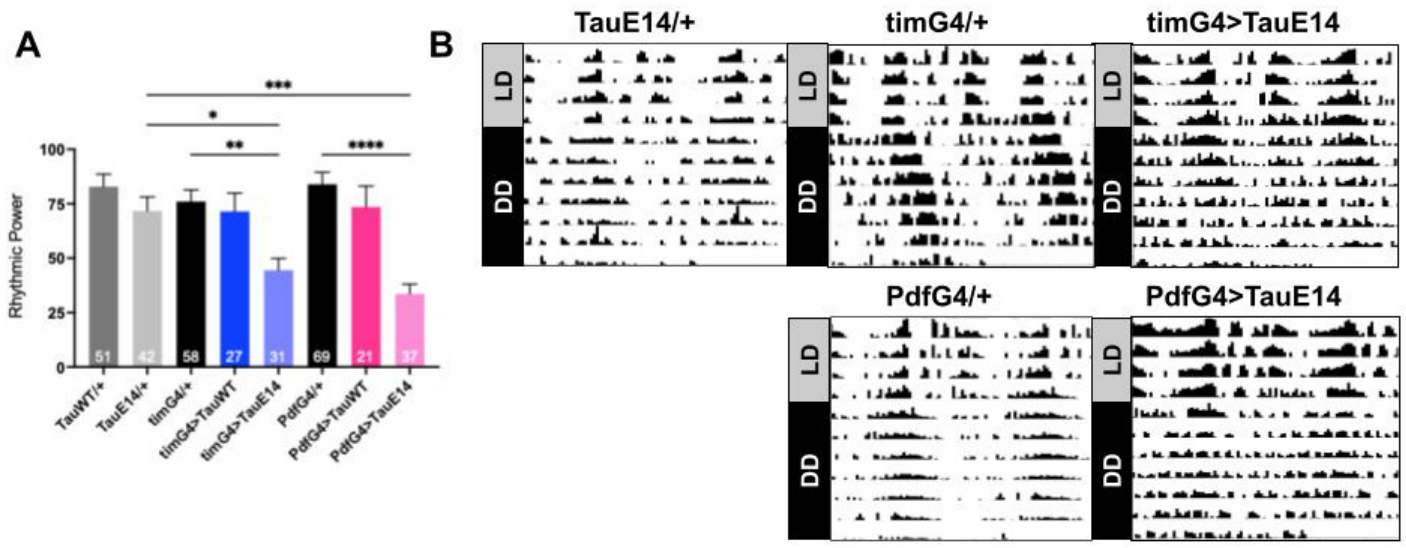

Fig. 6. PhosphoTau (TauE14) expression suppresses circadian behavior. We observed a reduction of rhythmic power using the Drosophila Activity Monitoring system and chi-square periodogram analysis.

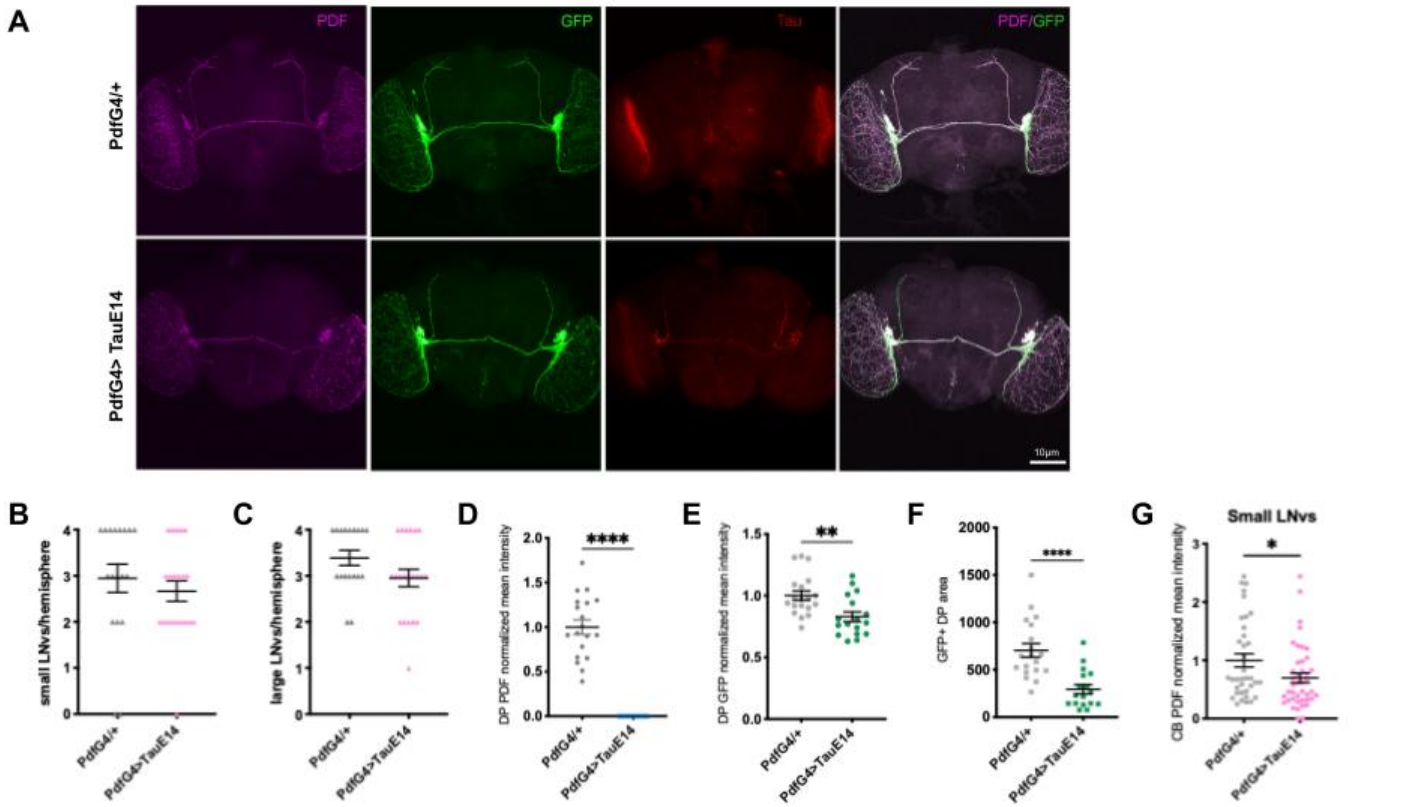

Fig. 7. TauE14 disrupts axonal transport of the neuropeptide PDF. We imaged and quantified PDF neuropeptide using confocal microscopy in flies expressing Tau E14 and noted a reduction in axonal PDF levels.

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.** 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

7. **PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS**
    - **What individuals have worked on the project?**

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

Funding Support:	*This award*

Name:	*Bart Van Alphen*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	10
Contribution to Project:	*Dr. Van Alphen analyzed behavioral and genetic effects of TBI*
Funding Support:	*This award*

Name:	*Ravi Allada*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	1
Contribution to Project:	*He supervised this project*
Funding Support:	*This award*

Name:	*Melanie Zhang*
Project Role:	*PhD Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	1
Contribution to Project:	*She characterized the effects of TBI related phospho-Tau*
Funding Support:	*This award*

Name:	*Elizabeth Williamson*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	6

Contribution to Project:	*Ms. Williamson was responsible for maintenance of animals, food production, and ordering of supplies for the project*
Funding Support:	*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?** Yes

  735135(PI: Allada), Simons Foundation(SFARI), Defining Behavioral Gene networks for Autism Spectrum Disorder Genes Using Sleep and Circadian Rhythms (pending to active)

  AARG-17-532626 (PI: Allada), Alzheimer's Association, Discovery of Novel Mechanisms by which Sleep Modulates AB Toxicity (active to past funding)

  1R21NS110420-01 (PI: Allada), NIH/NINDS, Discovery of Novel Pathways Mediating Huntingtin Neurotoxicity (active to past funding)

  W81XWH1810594 (PI: Allada), Department of the Army (USAMRAA), Discovery of Novel Therapeutics for Disordered Sleep in Fragile X Syndrome (active to past funding)

  W911NF1610584 (PI: Allada), Dept of the Army -- Materiel Command, Multisensory Integration by Circadian Clocks (active to past funding)

- **What other organizations were involved as partners?**
  - Nothing to Report

8. **SPECIAL REPORTING REQUIREMENTS**
   - Not applicable
9. **APPENDICES**
   - Not applicable