AWARD NUMBER: W81XWH-22-1-0217

TITLE: Discovery of In Vivo Molecular Pathways Mediating Tau-Induced Sleep and Circadian Disruption

PRINCIPAL INVESTIGATOR: Ravi Allada

CONTRACTING ORGANIZATION: Northwestern University

REPORT DATE: MAY 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command

Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MAY 2023	Annual	1APR2022 - 31MAR2023

4. TITLE AND SUBTITLE

Discovery of In Vivo Molecular Pathways Mediating Tau-Induced Sleep and Circadian Disruption

5a. CONTRACT NUMBER
W81XWH-22-1-0217

5b. GRANT NUMBER
W81XWH-22-1-0217

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Ravi Allada

E-Mail: 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 this reporting period, we have discovered ~60 RNAi candidates that reproducibly alter sleep and/or circadian rhythmicity when expressed in combination with phosphomimetic Tau (TauE14). We identified these candidates through behavioral screening of nearly 400 Drosophila RNAi strains that target homologs of human genes linked to neurodegeneration through GWAS and systems biology approaches. We have focused on 9 RNAi strains that suppress the behavioral rhythmicity deficits induced by expressing TauE14 in circadian clock neurons. Of these candidates we find that RNAi knockdown of histone deacetylase HDAC1 using two independent RNAi lines suppresses TauE14 rhythmicity defects, validating this gene as a potential mediator of Tau neuropathogenic effects. In parallel to this screen, we have also established a novel model of TauE14 sleep disruption for use in RNAi modifier screening. Our approach validates the use of high-throughput in vivo behavioral screening combined with human genomic studies to identify novel molecular pathways that mediate disease processes in the brain.

15. SUBJECT TERMS

Drosophila, sleep, circadian rhythms, neurodegenerative disease, Tau

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
U	U	U	UU	11	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** Disrupted circadian sleep-wake cycles are prominent features of multiple neurodegenerative tauopathies, and in some cases these behavioral disruptions precede disease onset. Here we utilize a novel, high-throughput in vivo screening model in *Drosophila* to assess the causal role of candidate Tau pathways from human GWAS and systems biology datasets in sleep and circadian behavioral disruption.

2. **KEYWORDS:** Drosophila, sleep, circadian rhythms, neurodegenerative disease, Tau

3. **ACCOMPLISHMENTS:**
   - **What were the major goals of the project?**

Aim: To discover and characterize novel molecular pathways that modulate in vivo Tau mediated pathogenicity on sleep and circadian rhythms.

Task 1. Primary and secondary RNA interference screen to identify modifiers of Tau-dependent behavioral deficits.

Task 2. Assessment of modifier effects on GAL4 activity as well as axonal Tau, PDF neuropeptide, and mitochondria

   - **What was accomplished under these goals?**
     - We used high-throughput behavioral screening to identify ~60 candidate genes that potentially mediate the effects of Tau on circadian rhythms and sleep (Fig 1- 2, Table 1).
     - We validated HDAC1 as an age-dependent suppressor of TauE14-induced rhythmicity disruption using independent RNAi reagents (Fig. 3).
     - We established an additional *Drosophila* model of TauE14-induced sleep disruption for use in RNAi screening (Fig. 4).

   - **What opportunities for training and professional development has the project provided?**
     - During the reporting period, Ph.D., M.S. and undergraduate students received training in *Drosophila* genetics, circadian rhythms and sleep analysis, as well as scientific presentation of results.

   - **How were the results disseminated to communities of interest?**
     - We have presented results to Northwestern University researchers at departmental and lab group meetings. We plan to disseminate our results to the broader scientific community through publication and conference presentations.

   - **What do you plan to do during the next reporting period to accomplish the goals?**
     - We will evaluate HDAC1 cellular and molecular pathways by determining the impact of HDAC1 RNAi on GAL4 activity, Tau expression, and axonal distribution of the PDF neuropeptide and mitochondria. We will also employ the novel GAL4-TauE14 sleep-disruption model for RNAi screening in order to identify modifiers of Tau-induced sleep phenotypes.

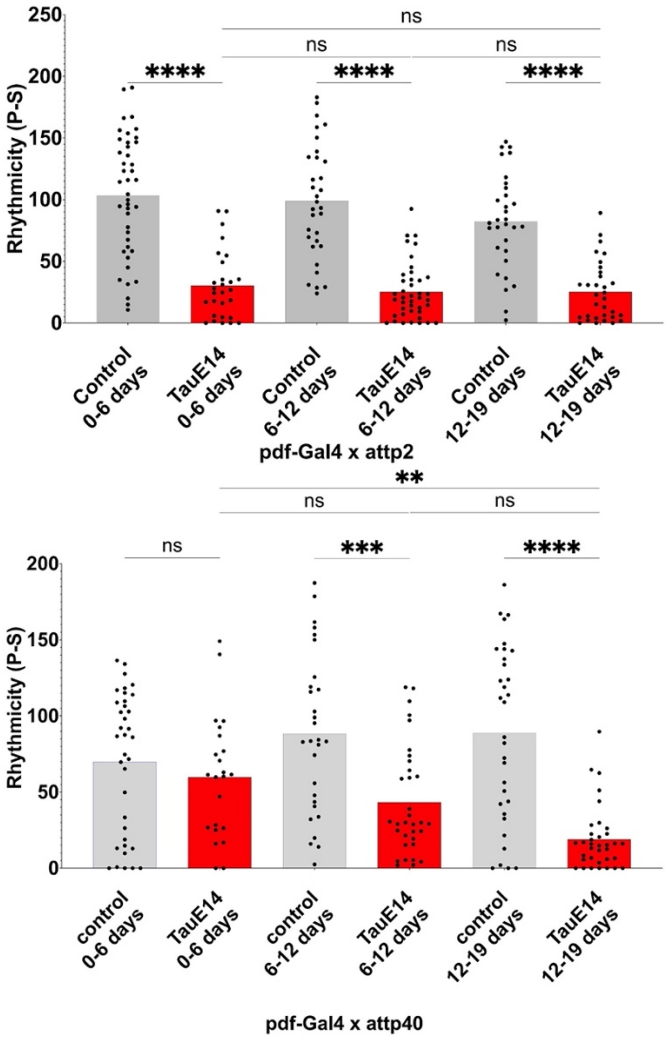

Figure 1. Expression of phosphomimetic human Tau (TauE14) in PDF clock neurons results in decreased rhythmicity influenced by age and genetic background. UAS-TauE14; pdfGal4 flies and +; pdfGAL4 controls were crossed to the attP2 (top panel) or attp40 (bottom panel) TRiP control strains and progeny were subject to sleep and circadian behavioral assays at different age ranges (0-6 days, 6-12 days, and 12-19 days). For attP2 outcrosses (top panel), TauE14 expression in all three age groups resulted in significant reductions in rhythmicity compared to the corresponding control (n=28-42). For attP40 outcrosses (bottom panel), both 6-12 day old and 12-19 day old UAS-TauE14 pdfGAL4 flies show significant reductions in rhythmicity compared to controls, while 0-6 day old UAS-TauE14 pdfGAL4 flies do not show a significant difference from controls (n=23-36). **** $p < 0.0001$, ***$p < 0.0002$, **$p < 0.002$, ns indicates non-significant.

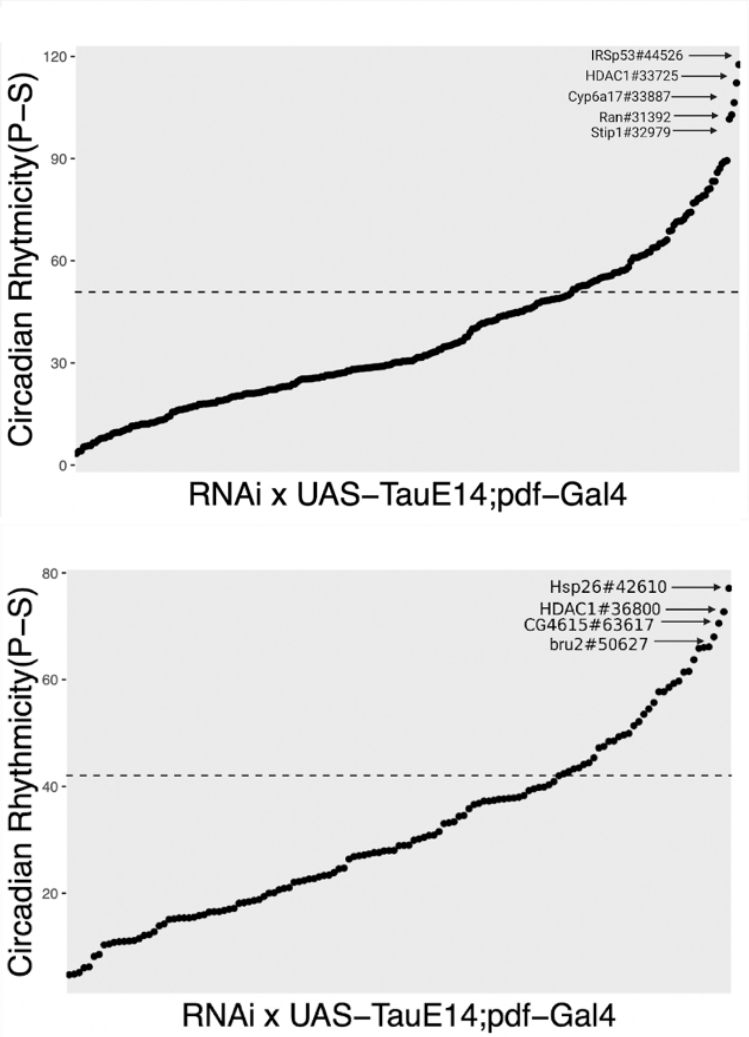

Figure 2. RNAi Screen Identifies Candidate Suppressors of TauE14 Reductions in Circadian Rhythmicity. Ranked plots of average rhythmicity (P-S) for RNAi strains crossed to UAS-TauE14;pdfGal4 flies. The top panel shows ranked P-S data for RNAi strains inserted into the attP2 locus (0-6 days old), while the bottom panel displays ranked rhythmicity data for RNAi strains in the attP40 locus (6-12 days old). For each plot, dotted line indicates the average P-S of the relevant age-matched control strain. In the top panel, attP2 RNAi strains showing the largest increases in rhythmicity relative to the control are IRSp53, HDAC1, Cyp6a17, Ran, and Hop(Stip). In the bottom panel, attP40 RNAi strains showing the strongest rhythmicity are Hsp26, HDAC1, CG4615, and bru2. n = 14-259.

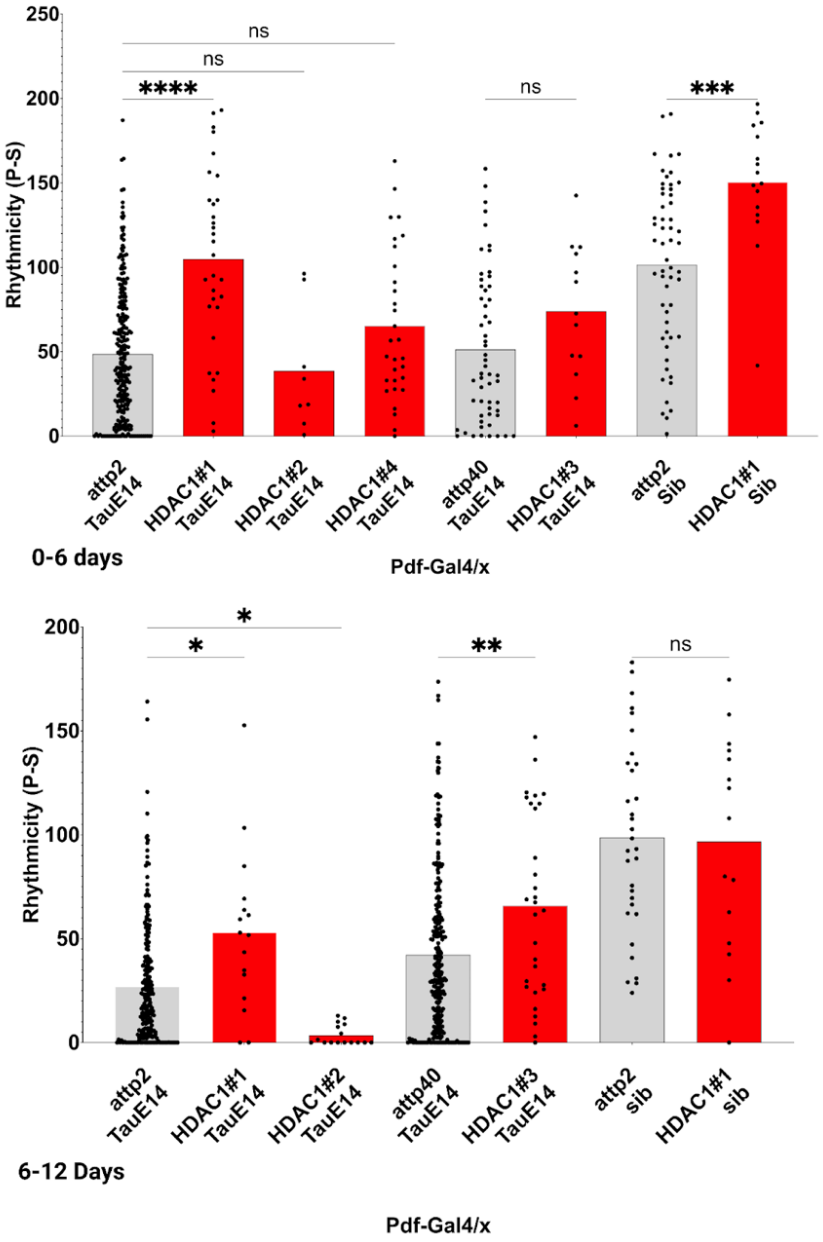

Figure 3. HDAC1 RNAi Suppression of TauE14 Mediated Arrhythmicity in 6-12 Day Old Flies is Tau Specific and Validated Using an Independent RNAi Line. Average rhythmicity of TauE14; pdfGAL4 flies with and without HDAC1 RNAi co-expression (top: first 6 columns; bottom: first 5 columns), and +; pdfGAL4 flies with and without HDAC1 RNAi (last 2 columns; 'sib' refers to +; pdfGAL4). Top panel: 0-6 days old; bottom panel: 6-12 days old. HDAC1 RNAi line #1 (#33725; attP2) promotes significant increases in rhythmicity in pdfGAL4 TauE14 flies compared to the attP2 control at both 0-6 days old (top panel) and 6-12 days old (bottom panel). HDAC1 RNAi line #1 also promotes a significant increase in rhythmicity in the absence of TauE14 in 0-6 day old (top panel, last two rows) but not in 6-12 day old flies (bottom panel, last two rows), indicating a Tau-specific effect in the older flies. HDAC1 RNAi line #3 (36800; attP40) also promotes significant increases in rhythmicity in 6-12 day old flies compared to attP40 control (bottom panel) but not in 0-6 day old flies (top panel). HDAC1 RNAi lines #2 (#34846) and #4 (#31616) do not promote significant increases in rhythmicity at the ages tested. n=15-228. Grey columns = attP2 or attP40 control; red columns = RNAi; **** $p < 0.0001$, *** $p < 0.0002$, ** $p < 0.002$, * $p < 0.05$, ns indicate not significant.

Phenotype	High P-S	Low P-S	Long Period	Short Period	High Sleep/Day	Low Sleep/Day	High Sleep Bouts/ Day	Low Sleep Bouts/Day
attp2 primary hits	13	27	13	11	9	12	8	9
attP2 replicated hits	4	21	6	3	3	2	0	3
attp40 primary hits	8	14	7	12	6	5	7	4
attP40 replicated hits	2	9	2	4	1	1	0	0
Total primary hits	**21**	**41**	**20**	**23**	**15**	**17**	**15**	**13**
Total replicated hits	**6**	**30**	**8**	**7**	**4**	**3**	**0**	**3**

Table 1: RNAi Screening of Tau-pathway Candidates Identified ~ 60 Strains that Reproducibly Alter Rhythmicity or Sleep in PdfGAL4 UAS-TauE14 flies. 384 RNAi strains targeting *Drosophila* homologs of Tau pathway candidate genes were screened by co-expression using UAS-TauE14; pdfGal4. Screen data were separated based on the genomic insertion locus of the RNAi constructs (attP2 vs attP40), as this influenced baseline phenotypes (see Fig. 1). Four behavioral phenotypes were assessed: rhythmicity (P-S) and period from constant dark conditions, sleep amount and sleep consolidation (bouts/day) from 12hr light:12 hr dark conditions. Primary hits were defined as strains falling approximately within the top and bottom 5% for each phenotype and thus selected for replication experiments. Replicated hits were defined as those strains showing a statistically significantly difference from control using data compiled from both primary and replication experiments.

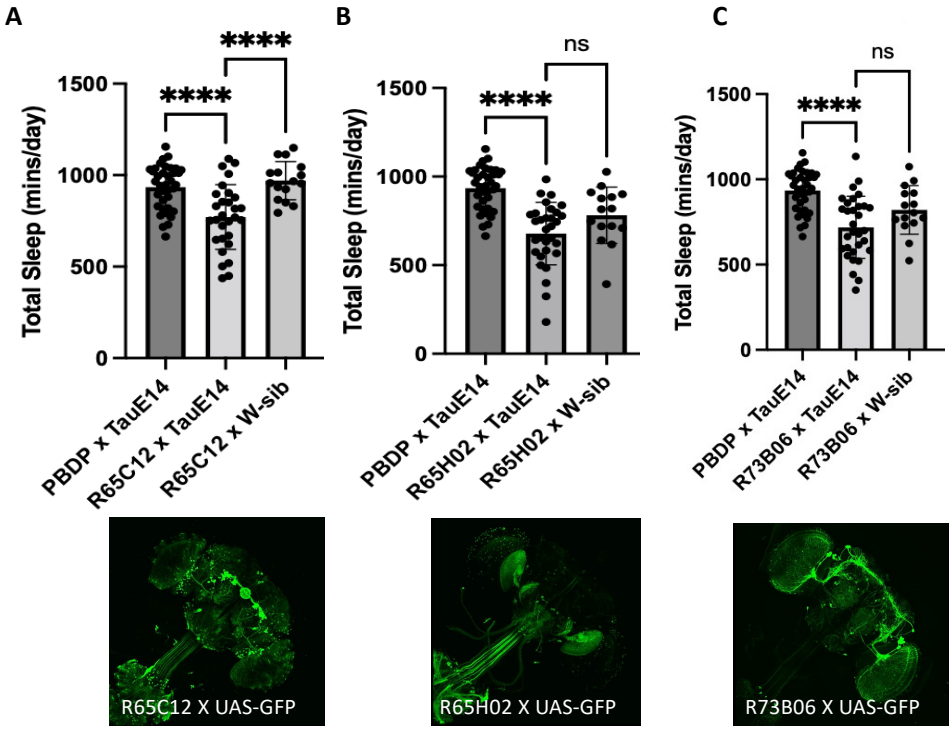

Figure 4. Establishment of *Drosophila* screening model for Tau-induced disruption in sleep. Due to fertility and viability issues in our initial sleep model strain (elavGAL4 UAS-Tau WT), we screened 78 GAL4 strains implicated in sleep regulation to identify GAL4 UAS-TauE14 flies exhibiting altered sleep levels. Top panels: we identified three GAL4/UAS-TauE14 strains that show a statistically significant and/or >100 minute decrease in sleep compared to both GAL4-only ('W-sib') and UAS-only (PBDP) control. Bottom panels:

Drosophila brain expression patterns of candidate GAL4 strains, which include putative sleep regulatory regions such as ellipsoid body (A; R65C12) and circadian clock neurons (C; R73B05) plus other brain regions (Jenett et al. 2012; doi:10.1016/j.celrep.2012.09.011)

Future Work:

We will follow up identified genes by retesting with independent genetic reagents and validate identified phenotypes. Candidate genes showing similar effects for both RNAi lines will be considered to be successful hits.

4. IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - The discovery of candidate genes mediating Tau effects on behavior provides insight into neurodegeneration mechanisms.
- **What was the impact on other disciplines?**
  - The identification of mechanisms of Tau neuropathogenesis may ultimately lead to novel treatments for tauopathies.
- **What was the impact on technology transfer?**
  - N/A
- **What was the impact on society beyond science and technology?**
  - If successful, new treatments for tauopathies could improve the lives of affected individuals.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
  - We have established a novel model for Tau-induced sleep disruption (Fig. 4) due to poor viability and fertility of the model (elavGAL4 UAS-TauWT) included in the original proposal.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
  - None during the reporting period
- **Changes that had a significant impact on expenditures**
  - None during the reporting period
- **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.**

    Zhang MY, Lear BC, Allada 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

  - **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:	*Ravi Allada*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-4371-1577*
Nearest person month worked:	*1*
Contribution to Project:	*Experimental design and supervision*
Funding Support:	*This award*

Name:	*Md Saheb Ali*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4*
Contribution to Project:	*Dr. Ali performed behavioral screening experiments.*
Funding Support:	*This award*

Name:	*Shiju Sisobhan*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Sisobhan performed quantitative analysis of behavior data*
Funding Support:	*This award*

Name:	*Gregory Wesseling*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*4*
Contribution to Project:	*Mr. Wesseling performed behavioral screening experiments*
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?

New Active Funding
Title: **Molecular and Cellular Basis of Sleep Homeostat**
Major Goals: The major goals are to identify sleep-wake cells that are necessary for wake-experience dependent sleep in adults, to identify sleep-wake cells that track prior waking experience, to identify genetic or biochemical changes that track sleep-wake experience in homeostats, and to identify genes that are necessary for wake-experience dependent sleep in adults
Project Number: R35NS132223
Name of PD/PI: Allada
Source of Support: NIH/NINDS
Project/Proposal Start and End Date: (MM/YYYY): 05/01/2023-03/31/2031
Total Award Amount (including Indirect Costs):

Funding Ended
Title: **Creating Comprehensive Maps of Worm and Fly Transcription Factor Binding Site**
Major Goals: The goal of this proposal is to finish ChIP experiments for the remaining Drosophila TFs for which data has not yet been produced.
Project Number: 11524//5U41HG007355-08
Name of PD/PI: Allada
Source of Support: Univ of Washington/NHGRI
Project/Proposal Start and End Date: (MM/YYYY) (if available): 10/01/2019-03/31/2023
Total Award Amount (including Indirect Costs):

Title: **Analysis of Ataxin2 Targets as Mediators of Amyotrophic Lateral Sclerosis**
Major Goals: The goal of this proposal is to examine mechanisms by which Ataxin2 impacts sleep and circadian rhythms using ALS models.
Project Number: 2022 Award
Name of PD/PI: Allada
Source of Support: Les Turner ALS Center
Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/01/2022-12/31/2024* (as lab is moving the grant ened prematurely)
Total Award Amount (including Indirect Costs):

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

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