AWARD NUMBER: W81XWH-22-1-0179

TITLE: Zebra Fish as a Model to Identify Genetic and Neuronal Mechanisms Through Which NF1 Regulates Sleep

PRINCIPAL INVESTIGATOR: David Prober

CONTRACTING ORGANIZATION: California Institute of Technology, Pasadena, CA

REPORT DATE: May 2023

TYPE OF REPORT: Annual

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

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE | 2. REPORT TYPE | 3. DATES COVERED |
|---|---|---|
| May 2023 | Annual | 01Apr2022-31Mar2023 |

**4. TITLE AND SUBTITLE**

Zebra Fish as a Model to Identify Genetic and Neuronal Mechanisms Through Which NF1 Regulates Sleep

**5a. CONTRACT NUMBER**
W81XWH-22-1-0179

**5b. GRANT NUMBER**
W81XWH-22-1-0179

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

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

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**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**

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder caused by mutation of the *neurofibromin 1 (nf1*) gene. This disorder is characterized by pigmented skin patches, specific types of tumors, and behavioral deficits that include disrupted sleep. Individuals with NF1 often report deficits in initiating and maintaining sleep at night, reduced sleep duration at night, and drowsiness during the day. Human and rodent studies suggest that disrupted sleep is likely to be a primary effect of loss of *nf1*, but the mechanisms that underlie this disrupted sleep are unknown. Larval zebrafish were recently established as a useful vertebrate model for studying mechanisms that regulate sleep, and in preliminary studies we found that *nf1* mutant zebrafish show sleep deficits similar to those observed in humans with NF1. This project will use larval zebrafish as a model system to identify genetic and neuronal mechanisms through which loss *nf1* function affects sleep.

**15. SUBJECT TERMS**

Neurofibromatosis type 1, zebrafish, sleep, genetics, neural circuits

| 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 |
| Unclassified | Unclassified | Unclassified | Unclassified | 8 | 19b. TELEPHONE NUMBER *(include area code)* |

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## 1. Introduction

Neurofibromatosis type 1 (NF1) is a genetic disorder caused by mutation of the *neurofibromin 1 (nf1*) gene. This disorder is characterized by pigmented skin patches, specific types of tumors, and behavioral deficits that include disrupted sleep. Individuals with NF1 commonly report deficits in initiating and maintaining sleep at night, reduced sleep duration at night, and drowsiness during the day. Human and rodent model studies suggest that disrupted sleep is likely a primary effect of loss of *nf1*, rather than a consequence of other phenotypes associated with the NF1 disorder. However, the mechanisms that underlie this disrupted sleep are unknown, and identifying these mechanisms will require experiments that use animal models of NF1. The zebrafish has recently emerged as a useful diurnal vertebrate model for uncovering genetic and neuronal mechanisms that regulate sleep, and there is extensive evidence that sleep mechanisms identified in humans and rodents are broadly conserved in zebrafish. In preliminary studies, we found that *nf1* mutant zebrafish, which recapitulate many aspects of the human NF1 disorder, exhibit sleep phenotypes that are strikingly similar to those observed in humans with NF1. This project uses zebrafish as a model to identify genetic and neuronal mechanisms that underlie disrupted sleep that is observed in *nf1* mutant animals. Our results may provide novel therapeutic targets to treat disrupted sleep in NF1, which may reduce the cognitive, behavioral and physiological symptoms associated with NF1.

## 2. Keywords

Neurofibromatosis type 1, zebrafish, sleep, genetics, neural circuits

## 3. Accomplishments

**What were the major goals of the project?**

**Aim 1. Major Task 1: Test hypothesis that pathways implicated in other *nf1*-dependent phenotypes are involved in *nf1* mutant sleep phenotypes.**
Subtask 1: obtain IACUC and ACURO approval. Months 1-3. 100% complete.
Subtask 2: perform epistasis analysis of zebrafish *nf1* mutants with pathways implicated in *nf1* signaling. Months 4-6. 30% complete.

**Aim 1. Major Task 2: Perform a targeted screen to determine if pathways previously implicated in regulating zebrafish sleep are required for *nf1* mutant sleep phenotypes.**
Subtask 1: Use genetics and pharmacology for *nf1* epistasis analysis. Months 6-12. 20% complete.

**Aim 2. Major Task 1: Identify neurons that are activated or inhibited in *nf1* mutant zebrafish.**
Subtask 1: perform pERK immunohistochemistry. Months 13-15. 0% complete.
Subtask 2: perform *cfos in situ* hybridization. Months 16-18. 10% complete.
Subtask 3: perform GCaMP6f whole-brain imaging. Months 19-24. 0% complete.

**Aim 2. Major Task 2: Test the hypothesis that *nf1* mutants sleep less at night because *nf1* is required for serotonin signaling.**
Subtask 1: Perform epistasis analysis for *nf1* and the serotonergic raphe. Months 13-18. 20% complete.

**Aim 2. Major Task 3: Identify neuronal mechanisms that act downstream of *nf1* to affect sleep.**
Subtask 1: Perform perturbations on neuronal populations identified in Aim 2: Major Task 1 on *nf1* mutants. Months 18-36. 0% complete.

**What was accomplished under these goals?**

This project has two Specific Aims. In Aim 1 we proposed to determine the genetic mechanism through which loss of *nf1* results in sleep defects. In Aim 2 we proposed to determine the neuronal mechanism through which loss of *nf1* results in sleep defects. We have spent the first year of funding performing two main tasks. First, we worked to confirm the preliminary sleep data that was presented in the grant proposal. We have succeeded in confirming all of the preliminary results. Specifically, we found that *nf1a*-/-; *nf1b*+/- fish (hereafter referred to as

*nf1* mutants) sleep less at night due to shorter sleep bouts and increased sleep latency, show a decreased arousal threshold and reduced sleep depth at night, and sleep more early in the morning. We also confirmed the absence of defects in behavioral circadian rhythms in *nf1* mutants. Second, we have performed crosses of *nf1* mutants to a variety of other zebrafish lines that we will use to test specific hypotheses in Aims 1 and 2.

Specific major accomplishments during this reporting period include:

1. We successfully obtained IACUC and ACURO approval for all experiments.
2. We have performed zebrafish matings in order to obtain genotypes that are necessary for the experiments described in Aim 1: Major Tasks 1 and 2, and for Aim 2: Major Task 2.
3. We have begun pharmacology experiments for epistasis analysis of zebrafish *nf1* mutants with pathways implicated in *nf1* signaling, and testing for genetic interactions between *nf1* and the raphe.

Progress has been slower than anticipated for two reasons. First, restrictions on lab work due to COVID during the first 6 months of the funding period hindered experimental work. These restrictions have now largely been resolved. Second, we have been struggling with reduced fertility in our zebrafish colony, which has significantly limited our ability to efficiently generate the zebrafish lines required for the proposed experiments. We are working to identify and resolve the cause of this issue, and we are expanding the critical *nf1* mutant lines that are required for all experiments in the project. While this has delayed our progress, we have been able to generate some of the key zebrafish lines, and I am optimistic that we will be able to resolve this issue soon.

**What opportunities for training and professional development has the project provided?**

Research: Trainees meet weekly with the PI to discuss their latest experimental results, any technical problems, and plans for future experiments.

Group meetings, seminars, and conferences: The Prober lab has a weekly lab meeting and bi-weekly journal club. Each week one member of the lab presents their work, at a frequency of approximately once every 3 months. Trainees give a detailed presentation of their research, including past accomplishments, current experiments, technical issues, and planned experiments. These meetings provide an excellent opportunity for trainees to practice their oral presentation skills. Trainees also attend weekly Caltech Biology Division seminars and bi-weekly Caltech Neuroscience seminars, as well as annual Biology and Chen Institute for Neuroscience retreats. Trainees also participate in Biolunch, a weekly seminar series where graduate students and postdocs present their research to the Caltech community, and Neurolunch, a monthly forum for students and postdocs to present their research to the Caltech neuroscience community. These forums provide excellent opportunities for trainees to improve their oral presentation skills, obtain feedback about their research from the Caltech community, and learn about the diverse research performed by other labs at Caltech. Trainees are also encouraged to attend at least one scientific conference each year, where they give either a poster or oral presentation. These conferences are valuable opportunities to improve presentation skills, receive feedback on their project, and develop relationships with other scientists.

Mentoring: I mentor each trainee on their research, career development, and oral and written communication skills. Trainees write the first draft of manuscripts describing their research, and I then provide guidance and feedback to help trainees to convey their ideas concisely and effectively. I also solicit assistance from trainees when writing grants in order to teach them grantsmanship skills. Trainees are also encouraged to attend networking sessions at conferences in order to establish relationships with scientists from a broad from a range of fields, including those with similar lived experiences.

**How were the results disseminated to communities of interest?**

Nothing to report. Results are not yet ready to present to the community.

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

During the second year of funding, we plan to:

1. Complete epistasis analyses of zebrafish *nf1* mutants with pathways implicated in *nf1* signaling.
2. Perform epistasis experiments to identify other pathways involved in the effects of *nf1* on sleep.
3. Perform pERK immunohistochemistry and *c-fos in situ* hybridization on zebrafish *nf1* mutants.
4. Explore potential links between *nf1* and the serotonergic raphe in sleep.

## 4. Impact

The project is still in an early stage and has not developed to the point where it may have a broad 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.

## 5. Changes/Problems

**Changes in approach and reasons for change**

Nothing to report.

**Actual or anticipated problems or delays and actions or plans to resolve them**

As described above, progress has been delayed for two reasons. First COVID-related restrictions hindered experimental work during the first 6 months of funding, but these restrictions have now largely been resolved. Second, we are experiencing reduced fertility in our zebrafish colony, which has reduced our ability to efficiently generate the zebrafish lines required for our experiments. We are working to identify the cause of this issue and we are taking steps to overcome it. Steps to diagnose and fix the problem include performing water quality tests, sending animals for pathology tests at the Zebrafish International Resource Center, optimizing the density of fish in each tank, expanding our fish stocks so that we can mate fish less frequently while still obtaining the desired number of embryos from matings, and raising new generations of zebrafish lines more frequently in order to increase the number of fish that are at the optimal age for mating. While this issue has delayed our progress, we have been able to generate some of the key zebrafish lines, and I am optimistic that we will be able to resolve this issue soon.

**Changes that had a significant impact on expenditures**

Nothing to report.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, or select agents**

Nothing to report.

## 6. Products

Nothing to report.

## 7. Participants & Other Collaborating Organizations

| | |
|---|---|
| Name | David Prober, PhD |
| Project Role | PI |
| Researcher Identifier | |
| Nearest Person Month Worked | 1.2 |
| Contribution to Project | Dr. Prober supervises all aspects of the project. |
| Funding Support (if applicable) | |

| | |
|---|---|
| Name | Grigorios Oikonomou, PhD |
| Project Role | Staff Scientist |
| Researcher Identifier | |
| Nearest Person Month Worked | 9.0 |
| Contribution to Project | Dr. Oikonomou has been performing matings in order to generate the necessary zebrafish lines, and has been performing behavioral experiments. |
| Funding Support (if applicable) | |

| | |
|---|---|
| Name | Tasha Cammidge |
| Project Role | Research Technician |
| Researcher Identifier | |
| Nearest Person Month Worked | 6.0 |
| Contribution to Project | Ms. Cammidge is performing zebrafish matings, embryo collection, and fish husbandry, as well as genotyping in order to identify and raise fish of the desired genotypes. |
| Funding Support (if applicable) | |

| | |
|---|---|
| Name | Axel Dominguez |
| Project Role | Zebrafish Facility Technician |
| Researcher Identifier | |
| Nearest Person Month Worked | 3.0 |
| Contribution to Project | Mr. Dominguez performs fish husbandry, feeding, cleaning, general maintenance, and repairs needed to maintain the fish used for this project. |
| Funding Support (if applicable) | |

| | |
|---|---|
| Name | Jasmine Emtage |
| Project Role | Graduate Student |
| Researcher Identifier | |
| Nearest Person Month Worked | 4.0 |
| Contribution to Project | Ms. Emtage performed zebrafish behavioral experiments. |
| Funding Support (if applicable) | |

**What other organizations were involved as partners?**

Nothing to report.

8. **Special Reporting Requirements**

   **COLLABORATIVE AWARDS:** N/A

   **QUAD CHARTS (if applicable):** N/A

9. **Appendices**

   None.