Tracked Biotechnologies

Title

Sleep Fragmentation in Neuronal Aβ42-Expressing Drosophila Replication Study (Stage 1 Registered Report)

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Abstract

Sleep disruption is increasingly recognized as an early feature of Alzheimer's disease (AD). Recent work reported that neuronal expression of human amyloid beta (Aβ42) in Drosophila melanogaster induces fragmented sleep prior to cognitive decline. This Stage 1 Registered Report proposes a direct replication of this sleep fragmentation phenotype using an adapted TrackedFlyBox™ video-based monitoring platform. We will quantify total sleep, daytime and nighttime sleep, and sleep architecture metrics including bout number and bout length in 2–3 day old adult flies across Aβ42-expressing and control genotypes. We hypothesize that neuronal Aβ42 expression leads to increased sleep fragmentation, characterized by increased bout number and reduced bout duration. This replication will provide high-quality evidence supporting fragmented sleep as an early biomarker of AD pathology.

Fig 1. Graphical abstract for proposed project. AB42 flies along with rescue conditions will be generated and phenotyped in the TrackedFlyBox™ system.

Lay Summary

In this registered report, we will test whether flies engineered to express a human Alzheimer's disease protein show disrupted sleep. By continuously monitoring fly movement with automated

video tracking, we will measure whether these flies sleep in shorter, more fragmented bouts. The results will help validate sleep disruption as an early sign of Alzheimer's-like pathology.

1. Introduction

1.1 Research question, background, and relevance

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid beta accumulation, synaptic dysfunction, and cognitive decline. Mounting evidence suggests that sleep disruption may precede cognitive symptoms and may serve as an early biomarker of disease progression. Sleep fragmentation has been linked to altered amyloid clearance and neurodegenerative vulnerability.

Drosophila melanogaster provides a powerful genetic model to study neuronal mechanisms underlying sleep and neurodegeneration. Gerstner et al. (2024) reported that neuronal expression of human Aβ42 in flies induces fragmented sleep architecture, with increased sleep bout number and reduced bout length, prior to measurable cognitive impairment. Replicating this phenotype is critical for establishing robustness and reproducibility of sleep-based phenotypes in early AD models.

1.2 Hypotheses

The primary hypothesis of this study is that flies expressing neuronal Aβ42 will exhibit significantly more fragmented sleep compared with driver-only controls. Sleep fragmentation will be operationally defined as an increase in the number of sleep bouts across the 24-hour period, accompanied by a reduction in the mean duration of individual sleep bouts.

The primary outcomes of interest will therefore be (1) the total amount of sleep measured in minutes, (2) the total number of sleep episodes per 24 hours (split into day and night) and (3) the max length of sleep bouts, measured in minutes (split into day and night).

Secondary outcomes will include activity, region analysis, and feeding frequency.

1.3 Timeline

Data collection and analysis are expected to be completed within 5 months following Stage 1 acceptance, with Stage 2 submission immediately thereafter.

2. Methods

2.1 Experimental Design

This study will replicate the sleep fragmentation assay described by Gerstner et al. in adult Drosophila. Four experimental groups will be examined: flies expressing human Aβ42 (BDSC #33769) pan-neuronally under the elav-GAL4 driver (BDSC #458), dFabp flies (BDSC #15579), dFabp flies combined with UAS-Aβ42, and driver-only control flies (elav-GAL4/+). Each

experimental group will include approximately 35–40 male flies aged two to three days post-eclosion.

2.2 Randomization and Blinding

Flies will be randomly assigned to monitoring chambers using computer-generated allocation. Data processing scripts will be run with anonymized genotype labels, and group identities will remain blinded until completion of primary analyses.

2.3 Exclusion Criteria and Replacement

Flies will be excluded if they die during monitoring or if technical tracking failure occurs. Replacement flies will only be added prior to analysis to maintain target sample sizes.

2.4 Sleep Assay

Sleep will be monitored under standard 12:12 light–dark cycles using the adapted TrackedFlyBox™ continuous video platform. Sleep is operationally defined as ≥5 minutes of inactivity, consistent with established Drosophila sleep criteria.

2.5 Data Collection and Processing

Custom analysis scripts will extract inactivity periods and compute sleep metrics. Outputs will be formatted for direct comparison with Figure 4C–J of Gerstner et al. (2024).

2.6 Sample Size and Statistical Power

Sample size (35–40 flies per group) is based on the original study and provides ≥80% power to detect moderate effect sizes (Cohen's $d \approx 0.7$) at $\alpha=0.05$. Power calculations were performed using G*Power 3.1 (Faul et al., 2007). The minimum detectable difference corresponds to approximately a 20–25% change in bout length or bout number relative to controls.

2.7 Statistical Analysis Plan

Primary comparisons will test Aβ42-expressing flies versus driver-only controls. Bout number and bout length will be analyzed using two-sided t-tests or one-way ANOVA, depending on group structure. Secondary outcomes will be analyzed similarly. Holm correction will control family-wise error across outcomes.

2.8 Interpretation of Results

If neuronal Aβ42 expression induces fragmented sleep, this will strengthen evidence for sleep disruption as an early AD phenotype. Null findings would suggest limited robustness or moderating environmental/genetic factors, informing refinement of fly AD sleep models.

2.9 Dissemination of Information

All processed datasets and figures will be made publicly available through ResearchHub.

2.10 Study Status

At the time of Stage 1 submission, technical development has been completed and data collection is set to begin soon.

3. Other Required Information

3.1 Acknowledgements

The TrackedBio engineering and labeling team assisted with technical development of this project.

3.2 Funding

This work is funded by the ResearchHub Foundation. No other specific external funding was received for this study.

3.3 Author Contributions

M.P. conceived and designed the study, will perform experiments, analyze the data, and wrote the manuscript.

3.4 Ethical Approval

n/a

3.5 Conflicts of Interest

The author declares no conflicts of interest.

4. References

1. Gerstner JR, et al. Neuronal Aβ42 expression disrupts sleep prior to cognitive decline in Drosophila melanogaster. J Neurosci Res. 2024;102(3):455–468. doi:10.1002/jnr.25001.

2. Huber R, Hill SL, Holladay C, Biesiadecki M, Tononi G, Cirelli C. Sleep homeostasis in Drosophila melanogaster. Sleep. 2004;27(4):628–639. doi:10.1093/sleep/27.4.628.

3. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39(2):175–191. doi:10.3758/BF03193146.