The effects of Sleep Dysregulation on the progression of Alzheimer's disease in a fruit fly model

Rodrigo G Rubio*, Andrew Bran, Fabiola Amaya Reyes, Ellen Woo and David D. Lent
Department of Biology, California State University Fresno USA
*Presenting author, Corresponding author: dlent@csufresno.edu

INTRODUCTION

Recent evidence suggests animals sleep to maintain metabolic homeostasis in the CNS to avoid any neurotoxic substance build up (Xie et al, 2013). Evidence of sleep having a significant role in washing the brain may explain why sleep deprivation (SD) is linked to Alzheimer's Disease (AD) (Bishir et al, 2020). The reason why a vast variety of animals and humans sleep is to conserve energy by efficiently washing the brain.

Circadian rhythm is the physiological, behavioral, and neurological changes in a 24-hour time period automatically functioning and processing the essentials necessary for biological function. Flies with the period null (*per*^{0}) mutant have memory problems due to the genes cause of arrhythmic behavior. Wild-type flies with no sleep interference have greater memory consolidation compared to flies with the *per*^{0} gene (Fropf et al, 2018).

Tau proteins function to stabilize the structure of microtubules that act as bridges for nutrients and communication between neurons. In AD, tau proteins disassociate from microtubules and attach to one another to cause communication disruption between neurons and cause microtubules to collapse leading to tangles of proteins forming. Neurons cannot communicate with each other and die from lack of cellular transport, leading to neurodegeneration. More dissociative tau proteins spread to mutate other tau proteins and cause the disease to progress throughout the brain.

This study will discover if there is a correlation between sleep deprivation and tau protein pathology; one of the proteins responsible for causing AD. *Drosophila melanogaster*, fruit flies, will be used as a model system to better understand how sleep disruption affects tau pathology progression and subsequently impacts cognition.

Variables/Groups

For the learning and memory section of this experiment, the control group will also be separated into two groups: one group will be sleep deprived (Group B) while the other group will not (Group A). The Panneural-Tau group will be separated into two groups: one group will be sleep deprived (Group D) while the other group will not (Group C) (Figure 1.). All groups will be female virgin flies since the groups will be implemented for the hot maze trials and testing section (Figure 2.)

To compare the similarities between tau pathology and *per*^{0} gene, two groups will represent each mutated genetic line, Groups E and F respectively. Both groups will be male flies and be 1-5 days old. There will actively be a monitor using the DAM system and be in 12:12 light-dark conditions in an incubator at 25 degrees Celsius.

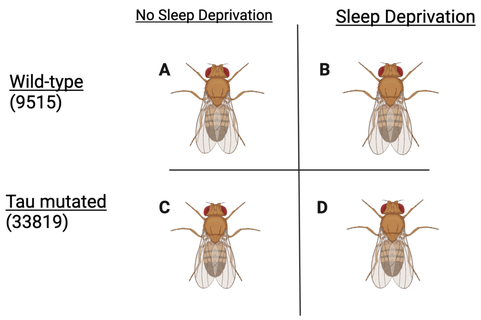

Figure 1. Four groups would be used in the experiment. (A) would be the control group of being wild-type with no sleep deprivation. (B) would be wild-type with sleep deprivation. (C) would have the MAPT gene expressed with no sleep deprivation. (D) would have the *MAPT* gene expressed with sleep deprivation. Groups B and D lifespans would be compared to determine if group D is dying directly from sleep deprivation. Virgin female flies will be used to conduct the learning and memory trials as their behavior is less socially aggressive compared to male fruit flies.

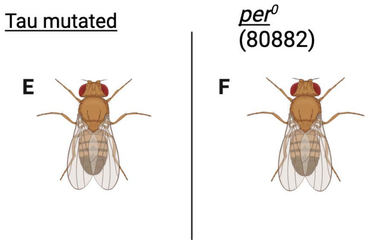

Figure 2. (E) *MAPT* gene expressed and (F) will have the *per*^{0} (arrhythmic) gene. Activity will be monitored by the DAM system to find any significant similarities between tau pathology and genetically mutated circadian rhythm.

METHODS

GAL4/UAS System

A simple method to express a specific gene that has been extensively used by scientists is the GAL4/UAS System (Figure 3.). This system requires two lines of flies: the "driver" line (contains the GAL4 gene) and the "reporter" (contains the UAS gene) line. The GAL4 gene is under control of a promoter gene, and the UAS gene controls the expression of the target gene. The GAL4 gene encodes for the GAL4 protein that binds to the UAS gene region to start transcription. The UAS gene line is next to the gene researchers want to study. In this experiment, the *MAPT* gene will be targeted to have an overexpression of tau proteins to cause a pathology in the brain region.

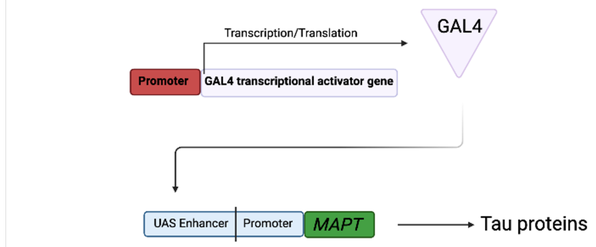

Figure 3. The male fruit will have the GAL4 gene and female fruit flies will have the UAS gene with the *MAPT* gene. The two lines will be crossed to cause an overexpression of tau proteins to lead to a pathology.

Spatial Learning and Memory

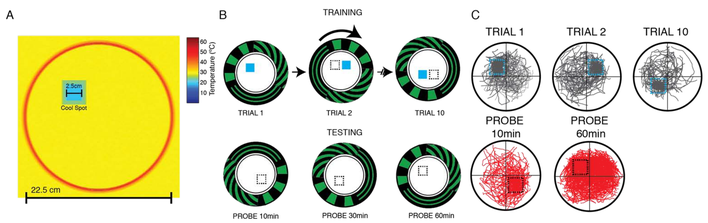

Figure 4. Visual image of the arena. A.) The thermal image view of the floor being heated with 64 thermoelectric squares while showcasing the cool spot for the flies to retreat to. Surrounding the Peltier array will be an LED display showing visual and spatial cues to the subject flies to reveal the cool spot location B.) The trial runs will involve the hot plate to cause urgency for the flies to find the cool spot with no object cue. The visual-spatial cues would allow the flies to be aware where the cool spot is located to train spatial navigation and visual memory. The test run would involve the flies to see the visual cues to retreat to cool spots. The amount of time the flies stay in the quadrant would be recorded. C.) Individual flies locomotion will be recorded and display with tracers to show majority time spent in a quadrant.

Sleep Depriving the Fruit Flies

It has been proven fruit flies can be sleep deprived from mechanical stimulation (Shimizu et al, 2008). This experiment will utilize an orbital shaker to sleep deprive the subjected fruit flies in different age groups: 1-5 days old, 10-15 days old, 30-35 days old. The fruit flies will be in 3 mm vials placed in a rack with sucrose based food supply (Figure 5.). The orbital shaker setting: orbital rotation speed: 20 and time: 40 seconds; reciprocal rotation angle and time : 60°, 60; and vibration setting is only applied for a five second pause. The total time of sleep disturbance is 2 hours, 11 minutes, and 15 seconds. Any sleep disturbances more than 3 hours may result in early death (Gilestro, 2012). The flies will be in the incubator during the sleep deprivation trials. Any fly that has died, their brains will be examined through microscopy. Trials will continue until all exposed flies have deceased.

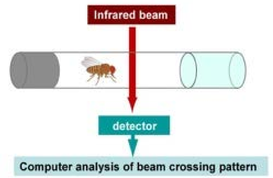

Figure 5. The mechanism of detecting activity from the fruit flies. There will be 32 flies individually placed in 5mm tubes. Groups E and F will be monitored separately in a 4 day period in 12:12 light-dark conditions to analyze sleep-wake activity. The total time the flies will be sleep disturbed is 2 hours, 11 minutes, and 15 seconds starting between 7:30pm to 9:00pm.

Histology

To visualize degeneration flies were prepared according to method described by Sunderhaus & Kretzschmar (2016). Histology images will be acquired using a QIclick camera mounted to a Leica DM2000 fluorescent microscope using a 10x or 20x objective lens. Differing layering sections will allow to detect any lesions in brain area.

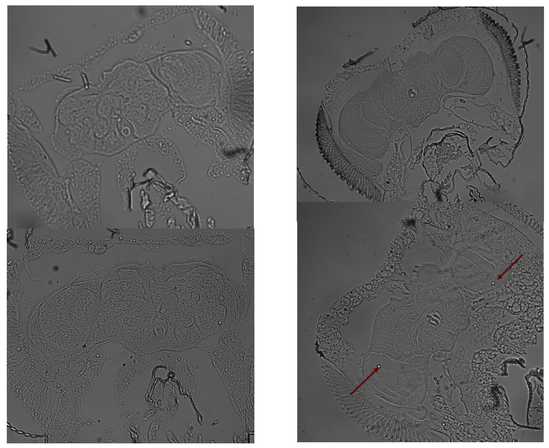

Figure 6: Four histological brain slides. Bottom right image showcase an example of lesions present.
Red arrows indicate vacuole.

Prediction:

The study expected an accelerated accumulation of tau expression in the fruit flies deprived of sleep. There should be an early death in the subjected fruit flies compared to the other groups. Regarding the learning and memory trials, the subjected fruit flies will decrease in time being spent in the cool spot.

ACKNOWLEDGEMENTS

- Sincere gratitude to Dr. Lent for mentorship and the opportunity to do this research.
- To the Department of Biology, College of Science and Mathematics and BB2P for partial funding of this research.