Melatonin's Effect on Learning and Memory in a Tauopathy Model of Alzheimer's Disease in *Drosophila melanogaster*

ABSTRACT

Alzheimer's Disease (AD) is a progressive neurodegenerative disease that causes devastating memory loss and cognitive decline in humans. There is no current cure for AD. Research studies show that oxidative stress is correlated to and possibly a cause of this neurodegeneration. Because antioxidants such as melatonin have been found to reduce oxidative stress, melatonin could alleviate neurodegeneration and serve as an effective dietary supplement for people with with AD. In this experiment, a tauopathy *Drosophila melanogaster* group that express human tau (MAPT) under gal4in neurons were used to model AD in humans. This study measured learning and memory of the *Drosophila* through an olfactory vortex learning assay in a t-maze. Groups of flies with and without melatonin supplementation were tested in the t-maze. Following experimentation and data collection, preliminary results from this study suggest, but do not confirm, that melatonin reduces memory loss and improves cognitive function in a *Drosophila* AD model. Further trials are needed to confirm the suggested results.

BACKGROUND AND RATIONALE

- Alzheimer's Disease (AD) affects > 5 million in USA and > 50 million worldwide
- Common form of dementia causes degenerative memory loss and cognitive decline
- Age-related disease; environmental/lifestyle factors can include: diet, exercise, sleep
- Neuronal Damage caused by:
  - beta-amyloid plaques
  - neurofibrillary tangles made of hyperphosphorylated tau protein (see right)
  - twisted microtubules = lack of neuronal nutrient transport leads to brain cell death
- Oxidative Stress = theory of aging = possible pathogenesis of AD
  - imbalance of reactive oxygen species (ROS) + endogenous antioxidants in body
  - ROS increase with aging and are correlated with AD progression
  - Oxidative stress is a likely cause of neurodegeneration, not side effect
  - Endogenous antioxidant defense decreases with age, therefore oxidative stress rises because nothing counteracts it
- Increased antioxidants counter increased ROS/oxidative stress
  - *Melatonin shows promising results for reducing neurodegeneration in models
- *Drosophila* = model for neurodegenerative diseases
  - share 70% of genes as humans + complex central nervous system (200,000 + neurons in brain)
- THIS STUDY:
  - tauopathy model expressing wild-type and mutant human tau in *Drosophila*
  - replicates neurodegeneration and rising oxidative stress

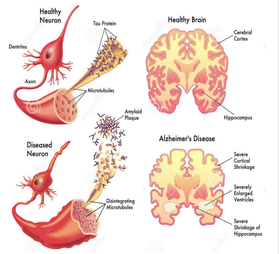


Figure 1. Normal versus hyperphosphorylated tau protein in Alzheimer's Disease brain.
Image from: https://healthjade.com/what-is-dementia/

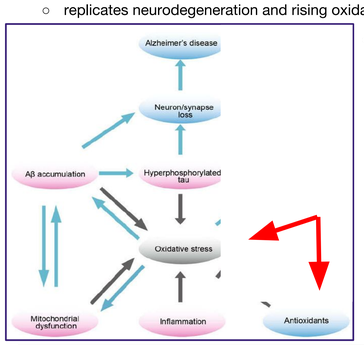


Figure 2. Pathway depicting relationship of oxidative stress and neurodegeneration/neuronal damage that occurs in Alzheimer's Disease.
Image taken from ResearchGate.net

- Olfactory Learning Assay to Negative Stimuli
  - vortex- and non-vortex paired scent = associative learning & memory
  - *Drosophila* navigate a T-maze and choose an odor
  - learning assays = more accurate reflection of neurodegeneration than measuring amount of tau present in this study

GOAL

The goal of this investigation is to determine whether melatonin supplementation can improve the **learning ability and memory** typically affected by Alzheimer's Disease using *Drosophila* models. Short- and long-term memory loss and reduced learning ability are caused by neurodegeneration that results from Alzheimer's Disease.

HYPOTHESES

Alternative [H_1]: A tauopathy model of Alzheimer's Disease in *Drosophila* on a melatonin-supplemented diet will learn, perform, and maintain long-term memory more consistently as measured by a olfactory learning assay than *Drosophila* raised on a normal diet.
Null [H_0]: A melatonin-supplemented diet will have no effect on learning and memory, as measured by an olfactory learning assay, of a tauopathy model of Alzheimer's Disease in *Drosophila*.

METHODOLOGY

A. Raising Flies

1. Standard fly media recipe taken from credible stock center; flies raised in vials in incubator set to 24° C
2. Genotypes: WT control; UAS-Gal4; Tauopathy under UAS-Gal4 control
3. Food: normal diet or melatonin-supplemented diet (100µg/mL dosage)

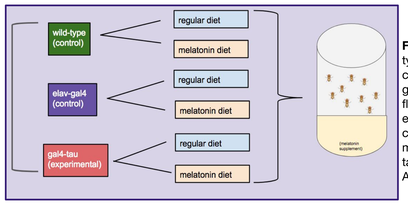
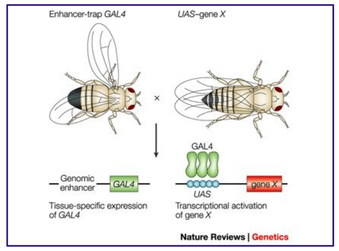


Figure 3 (left). Three types of flies used as control and experimental groups. White wild-type flies used as a control, elav-gal4 standard control, and gal4-tau fly model used as a tauopathy to replicate Alzheimer's Disease.

Figure 4 (right). Diagram of UAS-Gal4 system. (enhancer = ELAV gene X = human tau MAPT)
Image taken from igtrcn.org



B. T-maze Set-Up / Experimental Design

1. Gather pieces of t-maze for construction. The base and walls include:
   a. ½ inch acrylic pieces: Left Arm, Right Arm, T-Maze Base
   b. ¼ inch acrylic pieces: T-Maze Elevator and T-Maze Wall
2. Attach Left Arm of T-Maze to the Base about ¼ inch away from the center. Align Right Arm with Left Arm so that the two pieces are touching, and attach with a clamp or removable hold.
3. Insert elevator between Left and Right Arms so that large holes are aligned.

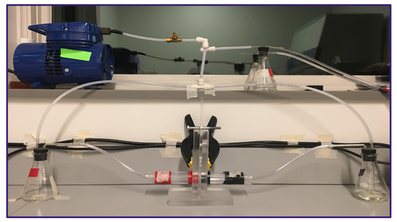


Image 1 (above). Setup for t-maze and training/testing circuit.

C. Training and Testing Flies

1. Standard olfactory shock assay protocol; substitute electric shock with vortex
2. Classical conditioning: 1 scent (CS+) vortex-paired and 1 CS-
   a. Train flies to associate each scent with negative stimulus
   b. Scents used: MCH and OCT (alternate CS+ scent each trial)
3. Test flies in t-maze both 5 minutes and 1 hour after training

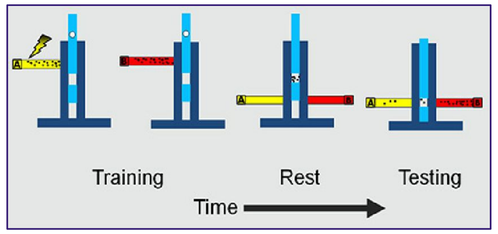


Figure 5 (above). Standard t-maze training and testing cycle for conditional earning assay.
Image taken from ResearchGate.net

RESULTS

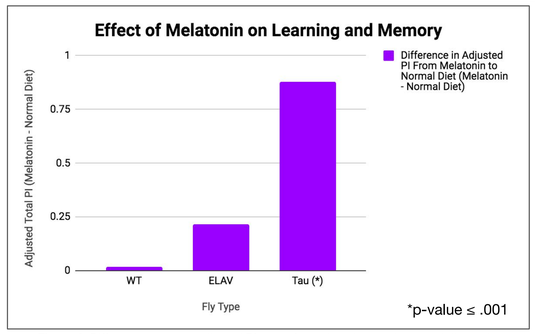


Figure 6. Difference between adjusted PI score for melatonin-supplemented diet and normal diet. All strains of flies on a diet with melatonin supplements, including tauopathy flies, showed an improved performance in the learning assay as compared to their counterparts not on melatonin.

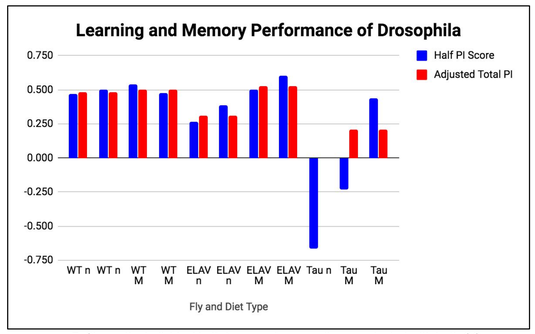


Figure 7. Shows the individual half PI scores for every fly group tested with both OCT and MCH as CS+. Adjusted = weighted to account for differences in sample sizes of each fly group. ELAV-gal4 M had best results followed by WT M. Data can vary between the same fly group.

Individual Learning And Memory Performance for Each Trial

Fly Type	Diet	CS+ Scent	Total # Flies	# Flies Correct (CS-)	# Flies Incorrect (CS+)
WT Control	Normal	MCH	15	11	4
WT Control	Normal	OCT	12	9	3
WT Control	Melatonin	MCH	13	10	3
WT Control	Melatonin	OCT	19	14	5
elav-gal4 Control	Normal	MCH	13	9	4
elav-gal4 Control	Normal	OCT	19	12	7
elav-gal4 Control	Melatonin	MCH	12	9	3
elav-gal4 Control	Melatonin	OCT	5	4	1
gal4-TAU	Normal	OCT	12	2	10
gal4-TAU	Melatonin	MCH	25	18	7
gal4-TAU	Melatonin	OCT	13	5	8

Table 1. A conditional learning assay was conducted to determine the learning and memory abilities of *Drosophila* at adult stages in their lives. Tauopathy flies experience neurological decline and suggested to perform better with melatonin supplements.

All images, charts, and graphs created by researchers unless otherwise stated.

DATA ANALYSIS

- Performance Index (PI) = how many flies chose correctly/incorrectly in t-maze
- Figure 2: melatonin supplementation improved the performance of all types of flies (.018 for WT → .877 tauopathy)
  - → correlation between melatonin supplementation and reduced memory loss/cognitive decline
  - likely through reducing oxidative stress and neurodegeneration in AD model
- WT flies = small difference in learning assay
  - suggests: melatonin supplementation does *not* have effect on learning and memory in healthy brain
- ELAV-Gal4 and tauopathy flies with melatonin showed *significant* improvement as compared to those w/o supplementation
- Tauopathy flies w/ and w/o melatonin performed worse on learning assay than other two types of flies
  - due to neurodegeneration that occurs in tauopathy model
  - melatonin *did* improve learning and memory, but cannot declare that it would reverse or stop progression of neurodegeneration
- Cannot conclude whether elav-gal4 flies' cognitive function changes depending on melatonin
- CS+/CS- has *no effect* on *Drosophila* performance

Statistical Analysis:

- standard deviation values:
  - WT group = .0884
  - elav-gal4 group = .2425
  - tauopathy group = .0661
- Z-test → p-value for three fly groups
  - WT and Elav-Gal4 > alpha-value of .05 ∴ lack of support against null hypothesis
  - Tauopathy flies < alpha-value of .05 (~.0002) ∴ reject null hypothesis and suggests to support hypothesis

CONCLUSIONS

Suggestions + Correlations:

- Melatonin supplementation improved the performance of all types of flies (wild-type and tauopathy flies)
- Correlation between melatonin supplementation and reduced memory and learning loss in tauopathy flies
- Reduced memory loss and learning impairment, likely through reducing oxidative stress and neurodegeneration in an AD model
- A small, if not insignificant, difference in wild-type flies receiving melatonin supplementation — slightly better performance than wild-type without supplementation
- More significant improvement in elav-gal4 and tauopathy flies with melatonin
- Neurodegeneration that occurs in a tauopathy → worsened performance of flies in learning assay
- Cannot conclude whether or not UAS-gal4 system affects any cognitive ability or decline in *Drosophila* in general
- Standard deviation — lack of support against null hypothesis for wild-type and elav-gal4
  - reject null hypothesis and suggests to support the hypothesis for tauopathy flies
- Z-test used and p-value determined to calculate significance

Simplifying Assumptions:

- Population is too small to make significant conclusions
- Adjusted PI values and p-value for tauopathy flies would be varied with more trials

FUTURE DIRECTIONS OF RESEARCH

1. Perform more trials of same experiment to determine validity/significance of trials
2. Test flies one hour after training to test long-term memory in addition to short-term memory
3. Test flies on day 1, 5, 10, 15, 20 to determine cognitive decline over lifetime as opposed to just once on day 5-7 — will show progression of Alzheimer's Disease in *Drosophila*
4. Divide flies receiving melatonin supplementation into:
   a. group with melatonin from birth
   b. group with melatonin starting at fully developed adult stage
   c. → determine whether age + level of neurodegeneration at which melatonin supplementation is introduced to diet will affect the efficacy of it and progression of AD
5. Add a second t-maze to modify training cycles and test more flies at one time