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1A. Research Plan
Category: Biomedical & Health Sciences

Title: Combating Familial Alzheimer's Disease: A Study of Resveratrol's Effects on a Presenilin Model of *Drosophila melanogaster*

Rationale:

Alzheimer's Disease (AD) is an irreversible, progressive neurodegenerative disease that destroys memory and learning. In specific, early-onset familial Alzheimer's Disease (FAD) is a form of early-onset AD that develops from genetic mutations. Although it is less common than late-onset AD, its significance and severity lies in that the genetic mutations that cause FAD are associated with the most aggressive form of AD (Sherrington et al., 1995). As the sixth overall leading cause of death in the United States (Atri, 2019), AD is the most common cause of dementia, or severe cognitive impairment. Furthermore, the cost for healthcare related to AD is estimated at nearly $500 billion annually (Weller & Budson, 2018). Given the serious and widespread effects of AD, further research on this disorder is a pressing need. To this end, AD is frequently studied through animal models; one such organism is *Drosophila melanogaster*, generally known as the common fruit fly. *Drosophila* is an ideal organism for the study of neurodegenerative diseases such as AD because it has conserved neurological function, a complex nervous system, and human disease-related loci (Stephenson & Metcalfe, 2013). The mushroom bodies (MBs) in specific are a key structure of the *Drosophila* brain that play a role in memory and learning. Past research has imaged the distinct structure of the MBs and encouraged studies that focus on their connection to brain function. Furthermore, presenilin *Drosophila* is a particular strain that has been used to model FAD. Immunofluorescent staining has been performed on dissected presenilin *Drosophila* brains in order to identify the MBs and ways that their structure is affected by FAD (McBride et al., 2010). The advantages of *Drosophila*, paired with current understanding of MBs and presenilin strains, makes *Drosophila* a valuable model for future AD research.

Finding effective medications is one focus of AD research. There are currently only five medical treatments, involving merely two classes of drugs, that have been FDA-approved to treat AD: three cholinesterase inhibitors (donepezil, rivastigmine, and galantamine), memantine, and memantine/donepezil. Additionally, these approved treatments are merely alleviate symptoms, rather than change the course of the disease (Yiannopoulou & Papageorgiou, 2013), and they have had a 99.6% failure rate based on past clinical trials (Briggs et al., 2016). In light of the need for better treatments against AD, one drug that has received attention is resveratrol ($C_{14}H_{12}O_3$), a polyphenol found in red grapes and wine. In particular, its anti-inflammatory properties and ability to mimic caloric restriction have been suggested as factors that may make it successful in combating AD. When resveratrol-treated mouse models of AD were used to test resveratrol's impact on amyloid beta plaques in the brain, it was found that the mice that were given resveratrol exhibited less plaques across several parts of the brain (Karuppagounder et al., 2009). Resveratrol has even been tested in clinical trials (although to a limited extent), and it was found to combat AD by stabilizing the progressive decline in cerebrospinal fluid Aβ40, plasma Aβ40, and cerebrospinal fluid Aβ42 levels (Moussa et al., 2017).

Studies suggest that resveratrol may be significant in helping combat AD, and these initial findings support a continued look into the potential of resveratrol. Finding new drugs is important given the severity of AD and the current lack of successful treatments, and resveratrol is the ideal drug to study given its proven safety and current data. This controlled study will use a *Drosophila* model of AD to identify the effect of resveratrol on memory and brain composition. It will also explore the effects of early exposure to resveratrol by performing identical tests on two generations of flies, one of which will

be given resveratrol after complete development and the other of which will be given resveratrol starting right after eclosion. The ultimate goal of this research is to determine whether resveratrol is a potential treatment option for AD patients.

Research Questions:

1. What is the effect of resveratrol on the memory of presenilin and wild-type *Drosophila melanogaster*?
2. How does resveratrol alter the brain composition of presenilin and wild-type *Drosophila melanogaster*, and what are the implications of these alterations?
3. How does the effect of resveratrol on the memory and brain composition of *Drosophila* differ when it is administered starting immediately upon eclosion?

Hypotheses:

1. Resveratrol will improve the memory of presenilin *Drosophila* but not have an impact on wild-type *Drosophila.*
2. Resveratrol will improve the brain composition (indicated by brighter images and more definition of the mushroom bodies) of presenilin *Drosophila* but not have an impact on wild-type *Drosophila*. This will indicate slower cell death and less neurodegeneration.
3. The effects of resveratrol will become even more apparent when resveratrol is administered immediately upon eclosion.

Engineering Goals:

1. To use the climbing assay to behaviorally test the memory of the flies
2. To dissect *Drosophila* brains, stain them with primary and secondary antibodies, then analyze them under the Keyence Fluorescence microscope.

Expected Outcomes:

For both 1st and 2nd generation flies:

1. Wild-type flies not given resveratrol will have a high success rate in the climbing assay and they will bright, defined cells in their brains.
2. Wild-type flies given resveratrol will have an equally high success rate in the climbing assay as the wild-type flies not given resveratrol, and they will have cells in their brains that are equally bright and defined as those of wild-type flies not given resveratrol.
3. Presenilin flies not given resveratrol will have a significantly lower success rate than the wild-type flies in the climbing assay and they will have darker and less defined cells in their brains.
4. Presenilin flies given resveratrol will have a significantly higher success rate in the climbing assay than the presenilin flies not given resveratrol (more similar to the rate of the wild-type flies) and they will have brighter and more defined cells in their brains than the presenilin flies not given resveratrol (more similar to the cells of the wild-type flies).

Procedure: Experiments Using a *Drosophila* Model

***Drosophila* Strains and Drug Administration: (Performed by student)**

1. Presenilin *Drosophila* will be used as the model of FAD, and 2u *Drosophila* will be used as the wild-type flies. All fly strains will be obtained from Indiana University's Bloomington *Drosophila* Stock Center.

2. Flies will be cultured in bottles at 25℃ in 60% humidity in a 12 hr:12 hr light:dark cycle, on standard cornmeal medium. 9 days after living in the bottles, the flies will be transferred into vials.
3. Two of the vials will be filled with 1.5 g of Fisher BioReagants Instant Fly Food, 5 mL of plain reverse osmosis (RO) water, and two gentle taps of yeast. The other two vials will also be filled with 1.5 g of Fisher BioReagants Instant Fly Food and two taps of yeast, but 0.4 g of resveratrol will be mixed into the 5 mL of RO water using a vortex mixer.
4. 30-35 2u flies will be put into one vial with plain RO water and one vial with resveratrol-incorporated RO water, and 30-35 presenilin flies will be put into the other two vials (Generation 1).
5. When the larvae and pupae become visible in the vials (Generation 2) after around a week, the grown flies will be transferred into fresh vials to make way for the flies that will newly hatch.

Climbing Assay: (Performed by student)

1. 16 days after eclosion (1 week after being in vials for the 1st generation flies), ten 1st Generation flies will be placed in a vial with a diameter of 2.5 cm and a height of 10 cm that has a mark at the 6 cm point.
2. The vial will be gently tapped to get the flies to the bottom of the vial, and the number of flies that climb to the 6 cm mark within 10 seconds will be counted. For each of the four fly groups, this process will be conducted for 3 batches of 10 flies each, and each batch of flies will undergo 3 trials.
3. The statistical significance between different genotypes and flies that received treatment will be calculated using One-way analysis of variance (ANOVA) for $P \leq 0.05$.

Evaluating antibodies for *Drosophila* brain staining: (Performed by student)

1. Six 1st Generation flies will be submerged in ethanol, immediately transferred to 1 X PBS, then immediately transferred into fixative, which will be prepared with 10 mL of 16% Paraformaldehyde, 4 mL of 10 X PBS, and 26 mL of milliQ water.
2. To dissect a fly brain in fixative, the head of the fly will be detached from the body using a pair of forceps.
3. The head capsule (including the eyes) will then be carefully ripped open and removed, isolating the brain.
4. Any remaining tracheae/other debris will be removed from the brain.
5. This dissecting process will be performed on at least 6 flies from each of the four groups, and after dissection, fly brains will be placed into a fresh well of fixative, with each fly group receiving its own well.
6. After 50 minutes had passed since the final brain was dissected, the brains will undergo three 5-minute washes in 150 uL of 1 X PBS; during each of the 5-minute intervals, the dissection wells will be put on a rocker in the lab room.
7. The brains will then be stained with Fasciclin II, placed on a rocker in the 4℃ cold room overnight, and stained with Gam the next day.
8. A microscope slide will be prepared by sticking two reinforcement labels on top of each other and dropping 16 uL of 80% glycerol into the center of the labels.
9. The fly brains will be transferred into the glycerol using a pair of forceps, then a coverslip will be gently placed on top of the reinforcement labels.
10. Clear nail polish will be used to seal the coverslip securely onto the slide.

11. Once the nail polish has tried, the slides will be observed under the Keyence Fluorescence Microscope.
12. Steps 1-14 will be repeated for each of the four fly groups. In the end, 4 total slides will be made (one for fly group), and each slide will have 6 fly brains.

2nd Generation: To understand the effect of earlier Resveratrol intake (Performed by student)

1. The 2nd generation flies will undergo the same steps as the 1st generation flies. However, the 2nd generation flies will be raised in vials on their experimental diets (including the administration of resveratrol for applicable groups) starting immediately upon eclosion, rather than after 9 days.

Risk and Safety:

Paraformaldehyde, which is one of the ingredients in the fixative that I will be using, poses the following safety risks: moderately toxic by skin contact (probably human carcinogen, may cause itch/rash, long-term exposure may cause reproductive/mutagenic problems); irritates nose and throat when inhaled (may cause lung damage); may react violently with strong oxidizing agents, ammonia, strong alkalis, isocyanates, paracids, anhydrides, and inorganic acids; vapors emitted are flammable.

Precautions that will be taken are that the fixative will be made inside the chemical fume hood and gloves will be worn both when making fixative and when performing brain dissections. Additionally, paraformaldehyde bottles will be disposed of in the closed paraformaldehyde waste container, which is located within the chemical fume hood.

Data Analysis:

A one-way ANOVA will be used to determine whether the data collected during the climbing assay is statistically significant. A separate one-way ANOVA will be conducted for the 1st and 2nd generation flies. Student t-tests will also be conducted in order to identify the relationships between specific groups, as opposed to the data as a whole.

Bibliography:

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Hazardous chemicals, activities, and devices:

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