Effects of Appl^{d} Mutation on the Olfactory System in Drosophila Melanogaster

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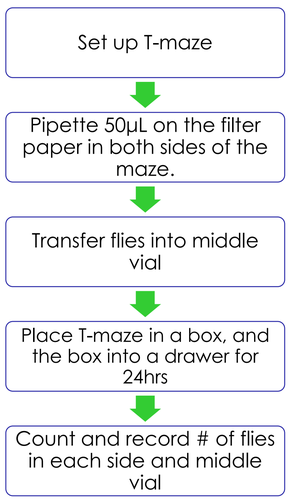
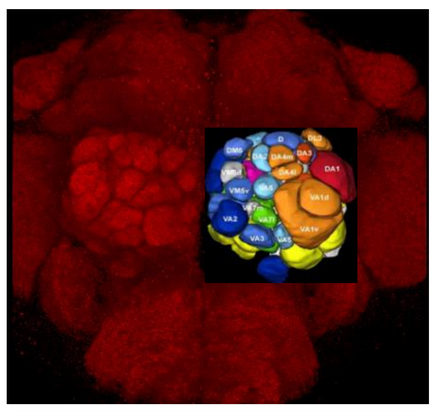
ABSTRACT

Alzheimer's disease (AD) is associated with mutations in the Amyloid Precursor Protein (APP). Previous research has shown that a deletion in the Drosophila ortholog APP-like (APPL) gene results in a lack of APPL expression. APPLd mutant flies show developmental defects and aging-dependent neurodegeneration similar to symptoms found in Alzheimer's disease. We hypothesize that APPLd mutants will show developmental defects in the olfactory system. My research project is to examine anatomical defects in the olfactory network of adult APPLd mutants and whether this translates into behavioral consequences in regard to their sense of smell. Using immunohistochemistry methods and confocal microscopy, we observed anatomical differences in the olfactory region (antennal lobe) in brains of young 2 days old APPLd mutants as compared to same-aged controls. This suggests that APPL may play a role during the development of the olfactory network. To test for their olfactory performance, APPLd mutant and wildtype fly's behavior was assayed using a T-maze experiment. We observed a significant difference in the response preference between mutant and wildtype fruit flies towards Apple Cider Vinegar (ACV). For wildtype flies, 50% of ACV has been established as the optimal concentration of ACV to elicit the largest attraction levels. By contrast, APPLd shows a higher preference for 75% than 50% ACV, suggesting an ameliorated repulsion effect. These results indicate that despite aberrant neuroanatomy, APPL fruit flies are able to smell but show subtle, significant changes in the functional properties of the olfactory system. We will test whether the observed defects of APPLd mutants are due to developmental effects or aging-dependent mechanisms. Future experiments will investigate whether the degeneration of the olfactory network is differentially regulated in aging APPLd mutants. Our research will provide insight into the fundamental role of molecules associated with Alzheimer's disease and may provide new pathways for therapeutic targets.

METHODS





Anatomy of a fly brain as revealed by immunostaining of synaptic proteins. Insert shows schematic of the glomeruli in the antennal lobe (fly olfactory system)

Preference Index (PI) $= \frac{\#\ of\ flies\ in\ ACV\ -\ \#\ of\ flies\ in\ water}{Total\ \#\ of\ flies\ in\ maze}$

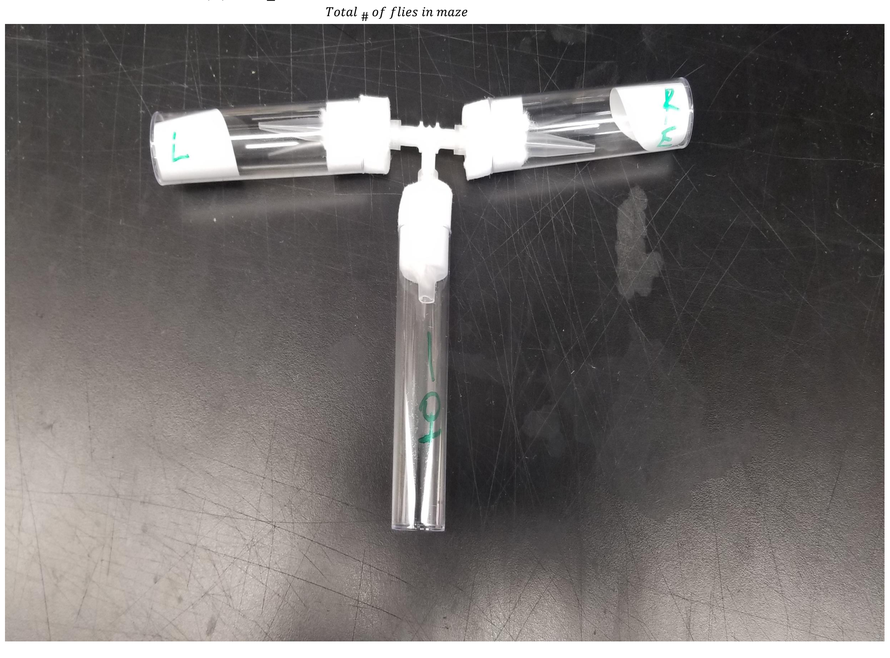


Figure 1: T-maze: The T-maze shown above is used to test for olfaction response to different concentrations of Apple Cider Vinegar (ACV). This behavioral assay utilizes pipette tips on each of its side to prevent the return of fruit flies back to the middle vial or out of the vial that was chosen first. Odors contained in the vial cannot escape into the environment, and as a result, the saturation is contained such that it is easily detectable by the fruit fly. The response rate and preference index can be calculated after the fruit fly has decided on which direction to follow.

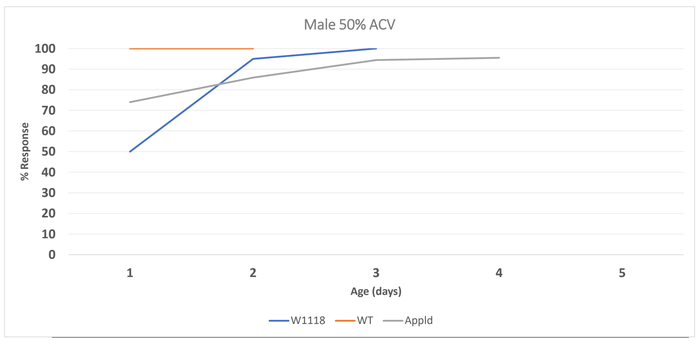


Figure 2: Behavioral assay results of Males from three different genotypes (W1118, WT, and Appld) at ages 1-5 days tested with 50% Apple Cider Vinegar (ACV) are shown. The Wild-type (WT) genotype shows a 100% response rate across ages 1-2d compared to the Appld genotype that shows an increase from ages 1-3d.

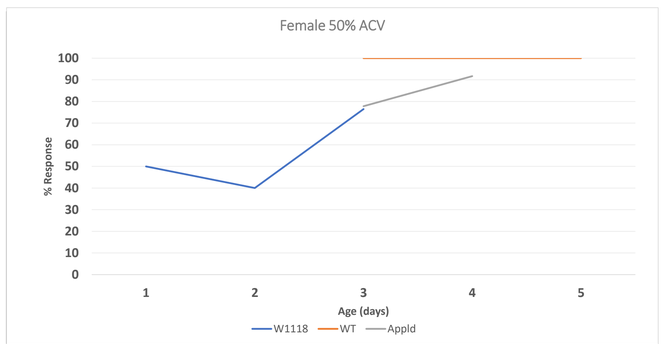


Figure 3: Behavioral assay results of Males from three different genotypes (W1118, WT, and Appld) at ages 1-5 days tested with 50% ACV are shown. The WT genotype shows a 100% response rate across ages 3-5d compared to the Appld genotype that shows an increase from ages 3-4d.

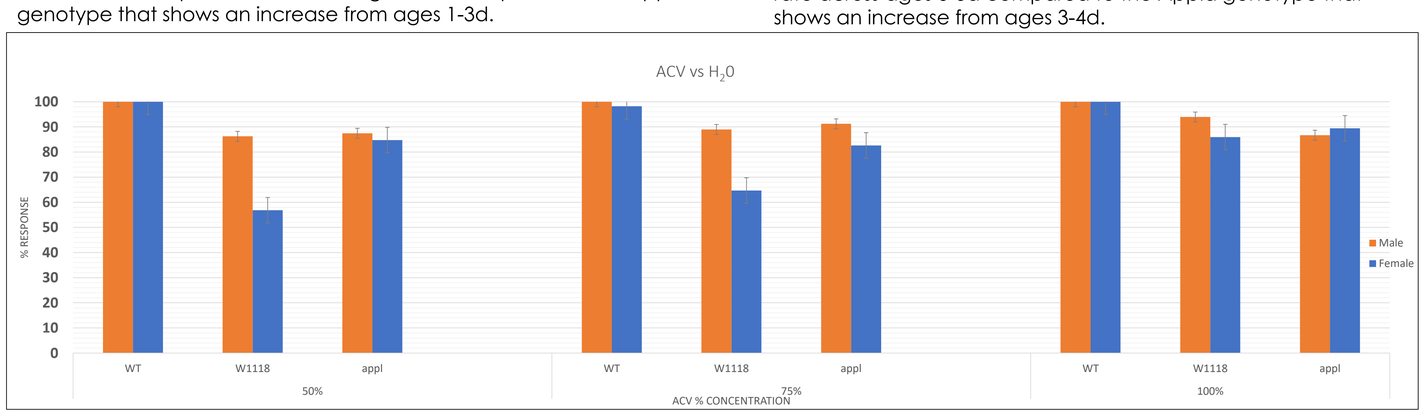


Figure 4: Behavioral assay results of WT, W1118, and Appld Male and Female response rate to 50%, 75%, and 100% concentration ACV. In most cases, males show a higher response rate than females. WT had the highest response rate of all three genotypes in both male and female fruit flies, which was close to 100%. By contrast, W1118 and Appld flies often show values below 90%.

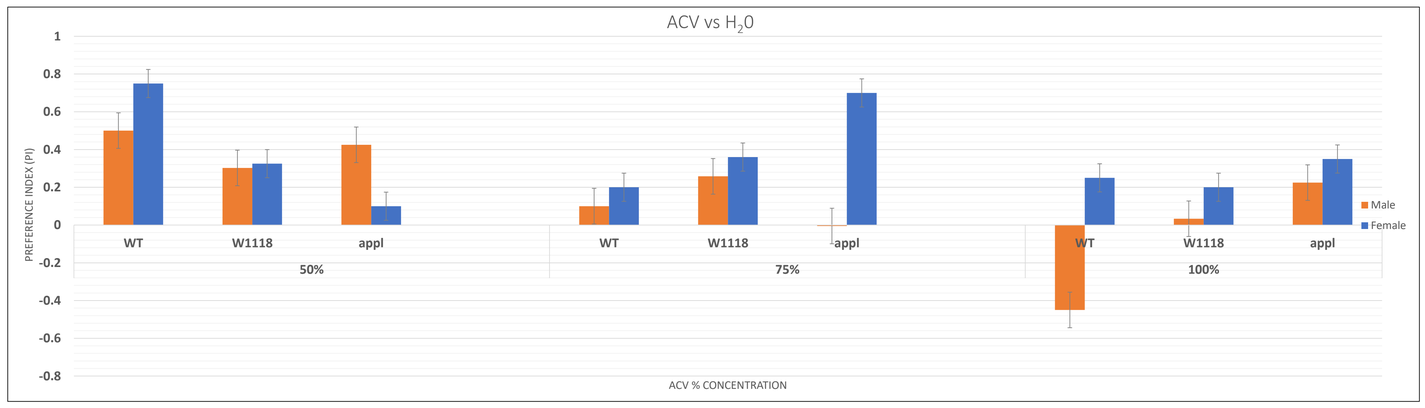


Figure 5: Behavioral assay results of WT, W1118, and Appld Male and Female preference index to 50%, 75%, and 100% concentration ACV. WT Male fruit flies preference index decreased as the ACV became more concentrated, and an aversion was recorded at 100% ACV. Appld Female fruit flies had an increase in its preference index as the ACV concentration increased from 50% - 70% but decreased at 100% ACV.

RESULTS

50% ACV was found as the optimal concentration to obtain the highest response rate in both Wild-type (WT) and Appld fruit flies.

As the WT fruit flies got older, no change in their response rate was observed. By contrast, Appld fruit flies showed an increase in response rate as the flies got older.

WT fruit flies, both male and female, responded at nearly perfect rate, whereas W1118 and Appld flies showed lower response rates.

Appld Male fruit flies had the lowest preference index out of all three genotypes at 75% ACV concentration, but females had the highest preference index of all three genotypes at the same concentration.

WT Male fruit flies were attracted to 50% ACV but were repelled at 100%. Appld males were not repelled at100% ACV.

WT Females showed a strong attraction to 50% ACV, which was not clearly observed in Appld females.

CONCLUSIONS AND FUTURE DIRECTIONS

Appld fruit flies have shown that they have some level of function in their olfactory system based on the percent response and preference index graphs. The next step is to figure out anatomically which one of their olfactory neurons in the olfactory network are degenerated and prevents full use of their olfactory system.

More experiments will be conducted on the three genotypes of fruit flies older than 5 days to learn more on how age affects response rate.

Appld flies will be dissected, and anatomically visualized to understand the role of gender in the neurodegeneration of the olfactory system.

ACKNOWLEDGEMENTS

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