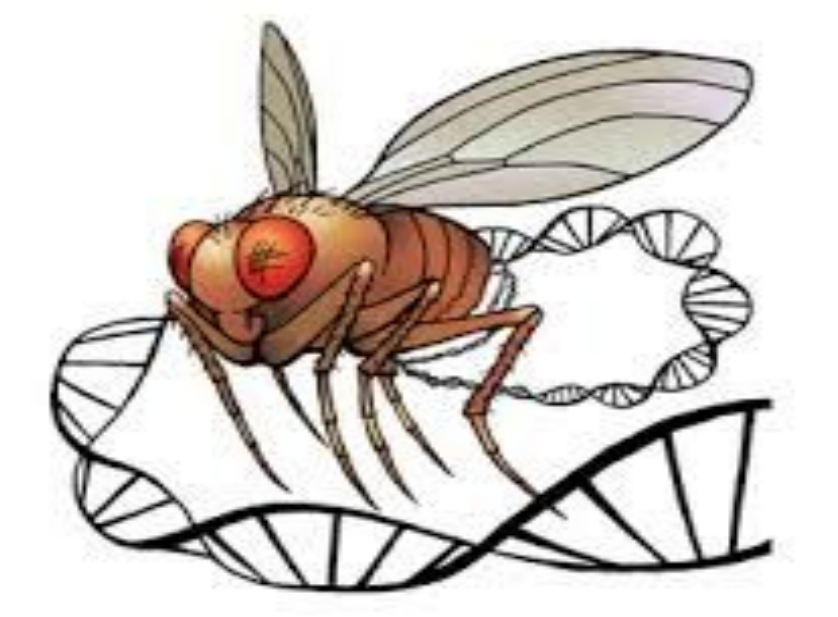# The Effect of Apoaequorin on Learning and Memory in *Drosophila melanogaster*

C. Jack Conway and Josie Gale; Stefanie Otto-Hitt, PhD
Department of Biological Sciences, Carroll College

Introduction

○ The organism *Drosophila melanogaster* (*D. melanogaster*) is a common model organism in biology.

○ Prevagen, which is primarily comprised of Apoaequorin, is a supplement that claims to improve memory.

○ Apoaequorin has calcium binding capabilities and protects against neuron degeneration.

○ The *Cadps* gene encodes activator proteins involved in the exocytosis of neurotransmitters and neuropeptides.

○ The *RyR* gene encodes receptors that regulate release of intracellular calcium.

○ **Hypothesis:** It was hypothesized that addition of Prevagen into the *D. melanogaster's* diet would improve learning and memory and increase expression of *Cadps* and *RyR* due to their role in calcium regulation.

Methods

○ **Culturing:** Flies were kept in 10 mL of potato flakes with 10 mL of DO water and five yeast pellets

○ **Treatment:** 30 flies were kept in 10 mL of potato flakes with 62 uL of Prevagen treatment solution and five yeast pellets

○ **Starvation:** Control and treatment groups were starved for five hours prior to performing behavioral assays

○ **Short-Term Memory Assay:** Flies were conditioned and placed in a Y-maze for seven minutes

○ **Long-Term Memory Assay:** Flies rested for 25 minutes before being placed into the Y-maze for seven minutes

○ **Anesthesia Resistant Assay:** Flies were cold-shocked for two minutes and placed into a Y-maze for seven minutes

○ **Gene Expression:** A combination of larval RNA extraction and RT-qPCR amplification was used to measure gene expression

Results

Tabel 1. Shows total number of flies for each replicate of each assay.

Week of Experiment	Control			Treatment		
	Short-Term Assay	Long-Term Assay	Anesthesia Resistant Assay	Short-Term Assay	Long-Term Assay	Anesthesia Resistant Assay
Week One (9/19)	5	5 (only 1 participant)	4 (only 1 participant)	5 (only 1 participant)	5	5
Week Two (9/26)	9	9	7	4	2 (only 2 participants)	1 (only 1 participant)
Week Three (10/3)	29	26	26	24	23	19
Week Four (10/10)	26	23	11	23	20	19
Week Six (10/24)	21	20	20	22	21	21
Week Seven (10/31)	22	22	22	29	29	29
Week Eight (11/7)	29	29	23	23	21	20
Week Nine (11/14)	26	24	22	29	22	21

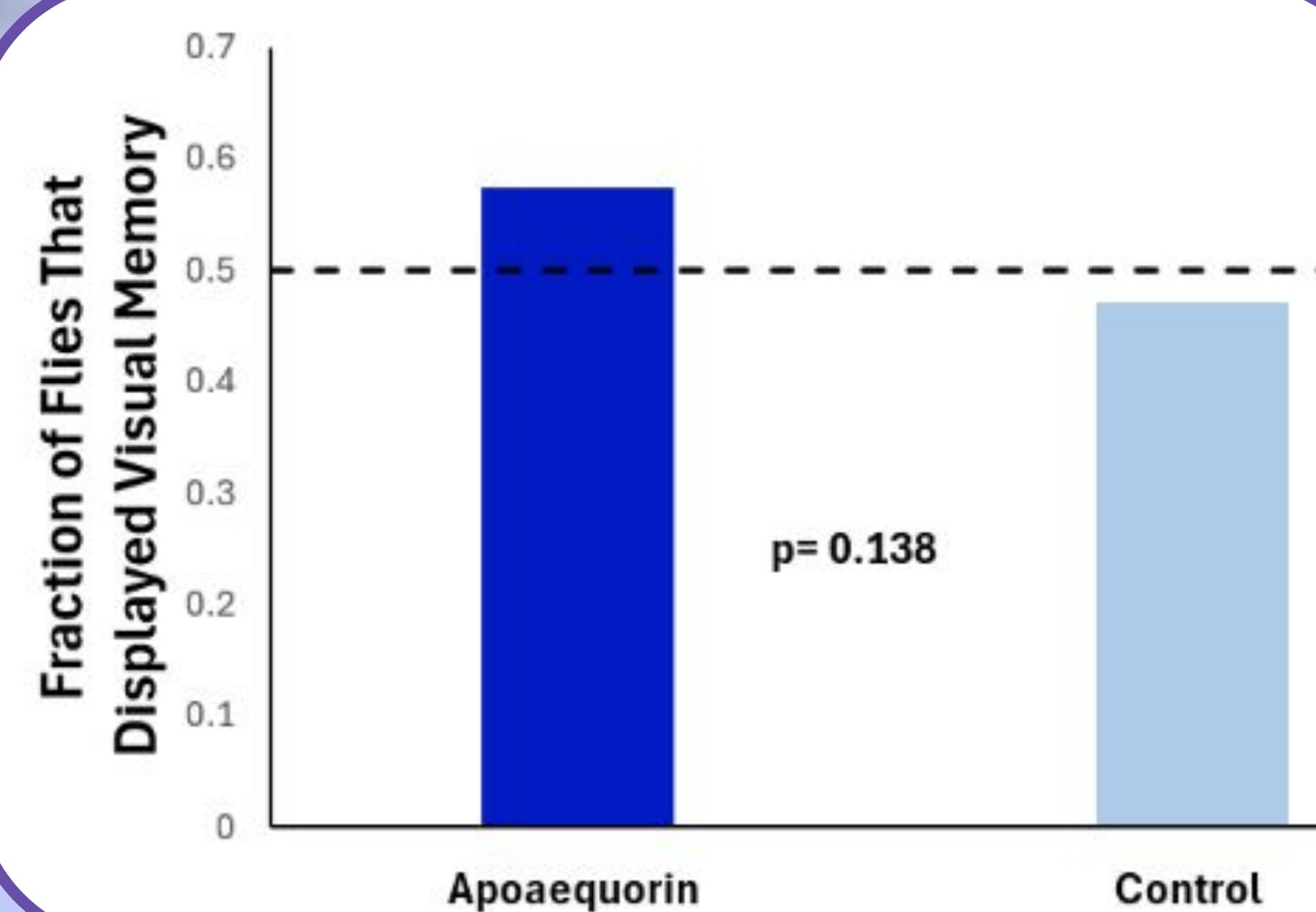**Figure 1.** Flies in a Y-maze undergoing decision point in memory assay

Figure 2. Short Term Memory Assay. Depicts the fraction of flies that made the correct choice following visual conditioning (p=0.138, n=110 & 104)
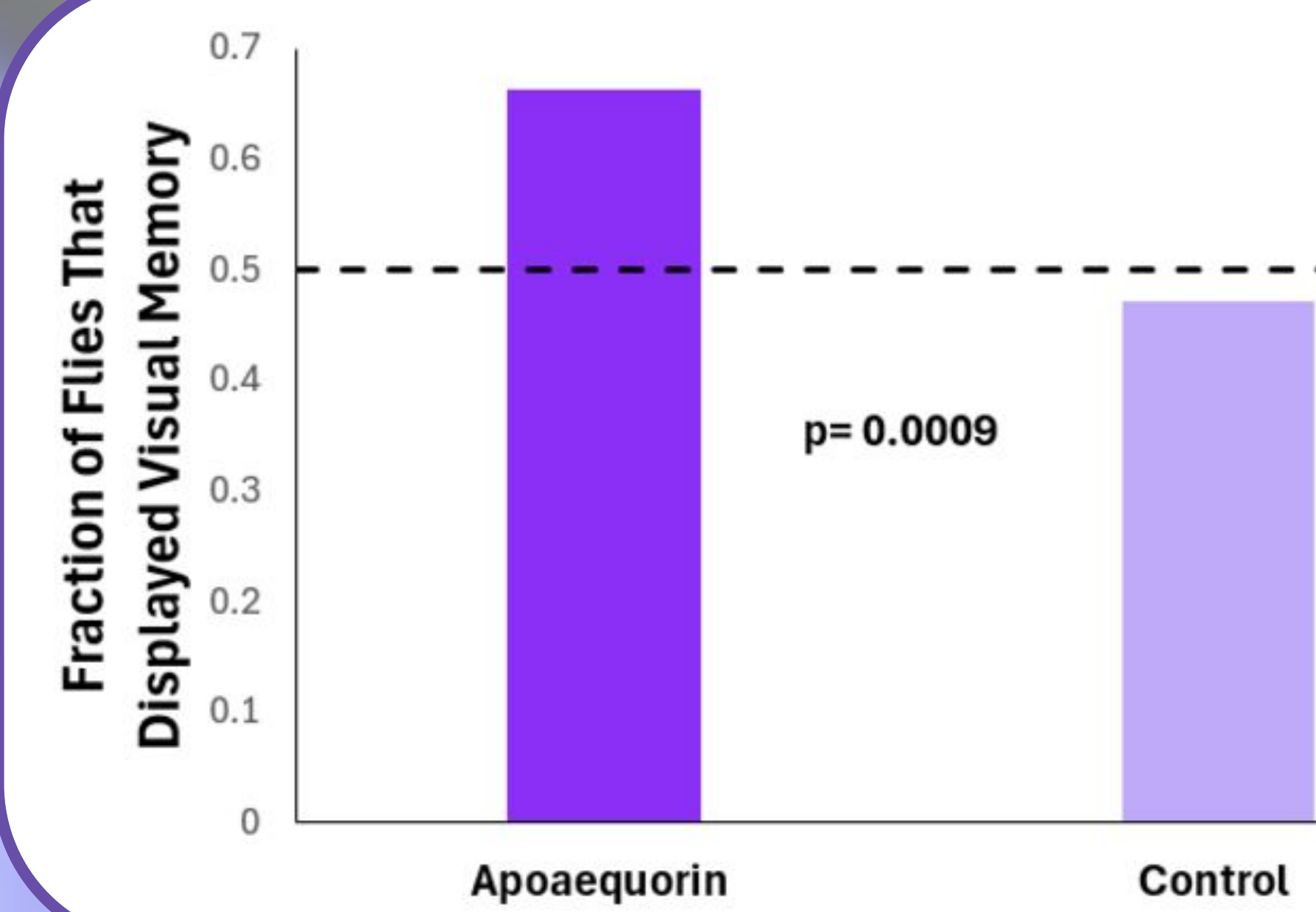

Figure 3. Long Term Memory Assay. Depicts the fraction of flies that made the correct choice following visual conditioning (p=0.0009, n=83 & 88)
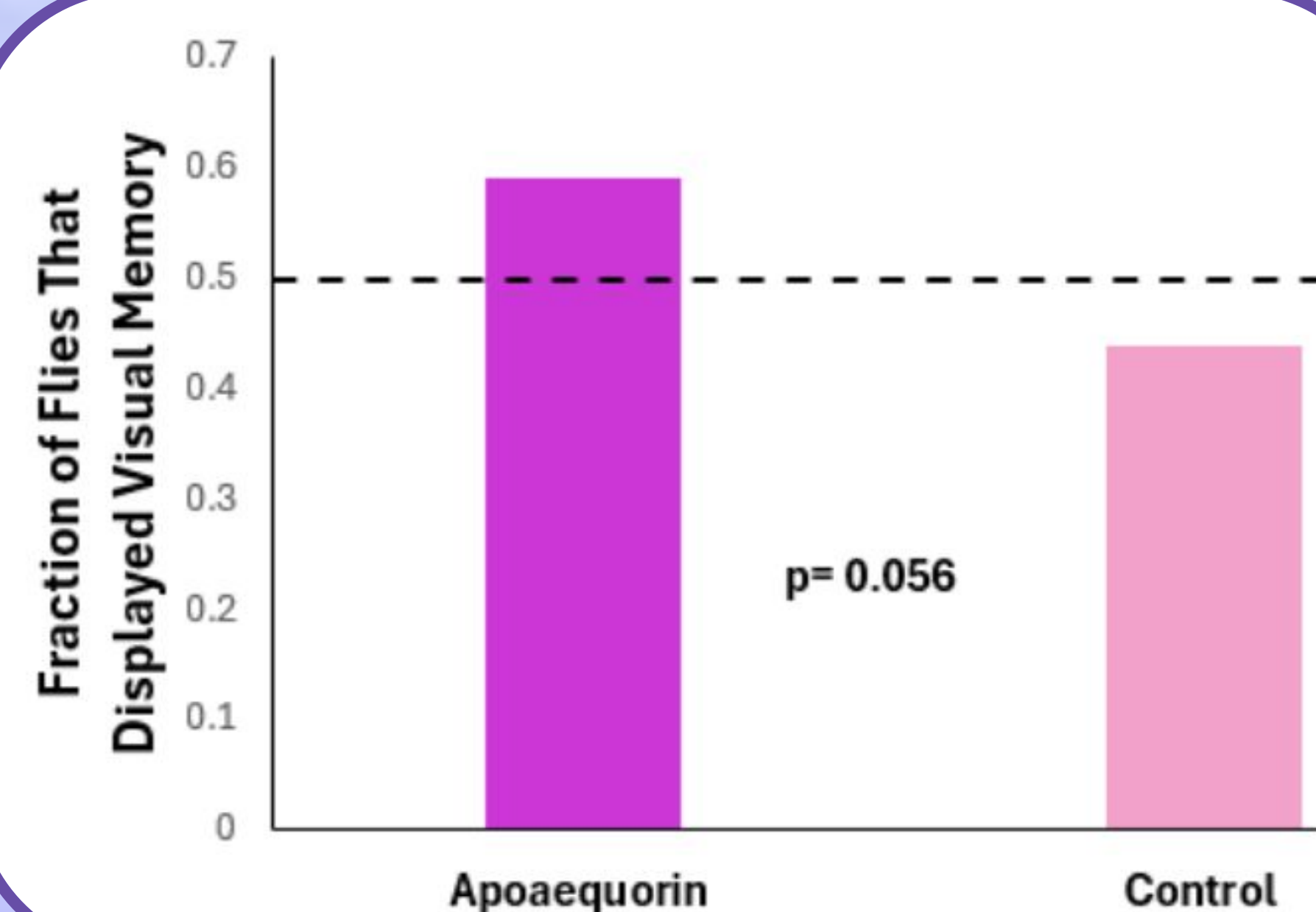

Figure 4. Anesthesia-Resistant Memory Assay. Depicts the fraction of flies that made the correct choice following visual conditioning (p=0.056, n=76 & 83)
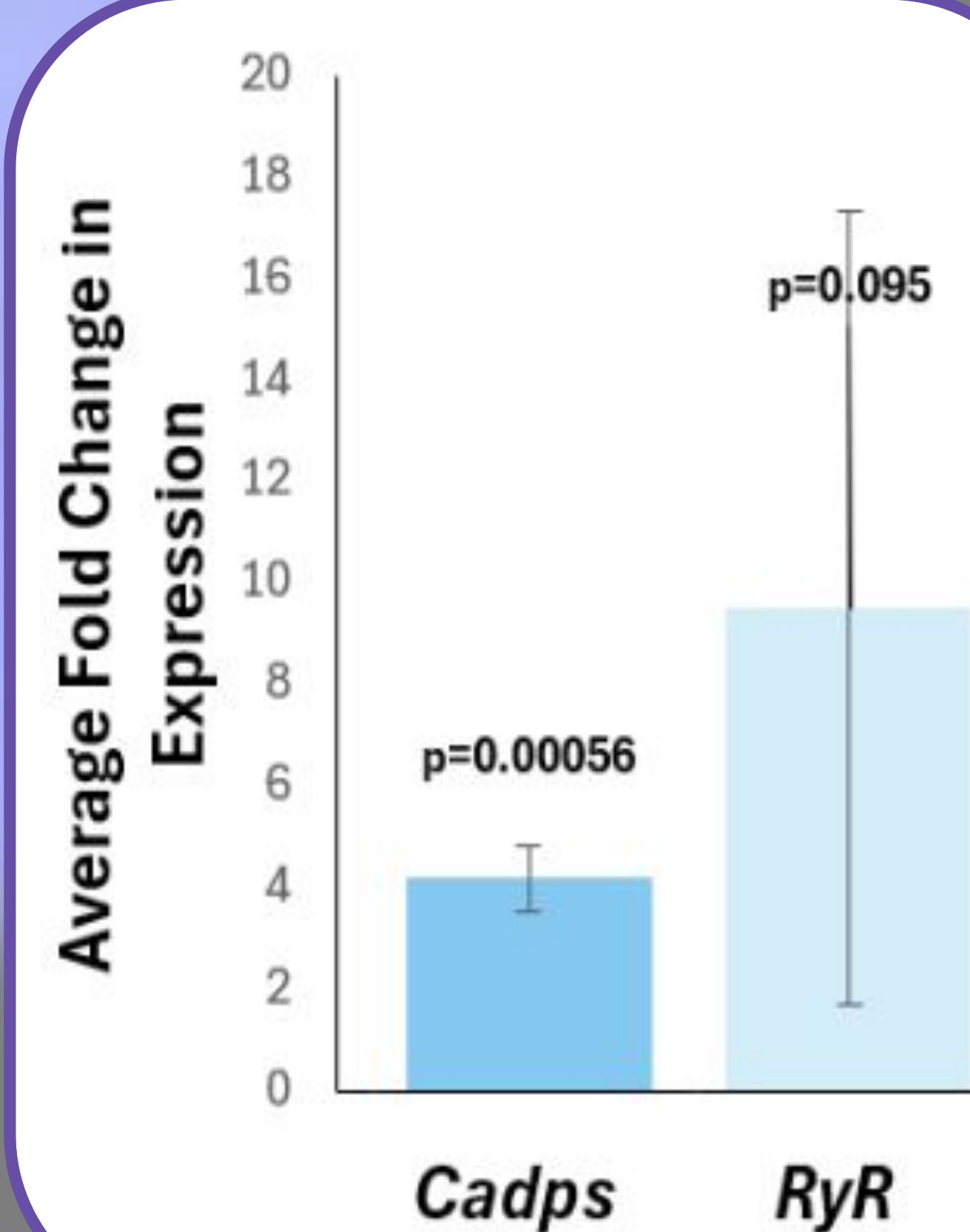

Figure 5. Fold change in expression of the *Cadps* and *RyR* genes in Apoaequorin-treated flies compared to control flies. Error bars represent the standard deviation of each mean (p=0.00056 & 0.095, n=5)

Conclusion

○ Apoaequorin treatment resulted in increased *Cadps* expression in *D. melanogaster* larvae.

○ Treated flies showed mixed learning and memory among the assays with only long term memory and learning showing significance.

○ Future research could target different genes, improve conditioning protocols for the memory assays, increase replicates, and increase sample size to limit variance.

○ The results show that, at a molecular level, Apoaequorin caused an increase in the expression of the *Cadps* gene and in learning and memory on a long term scale.

References

○ Ehlers VL. Brain Behav. 2020.
○ FlyBase. FlyBase Gene Report: DMEL\CADPS.
○ FlyBase. FlyBase Gene Report: DMEL\RYR.
○ Otto-Hitt S. 2024 Fall.
○ Quincy Bioscience. Prevagen®.
○ Shane-McWhorter L. Apoaequorin. Merck Manual Professional Edition. 2023.

Acknowledgements