



# The Effects of Alpha-GPC on Memory and Locomotion in *Drosophila melanogaster*

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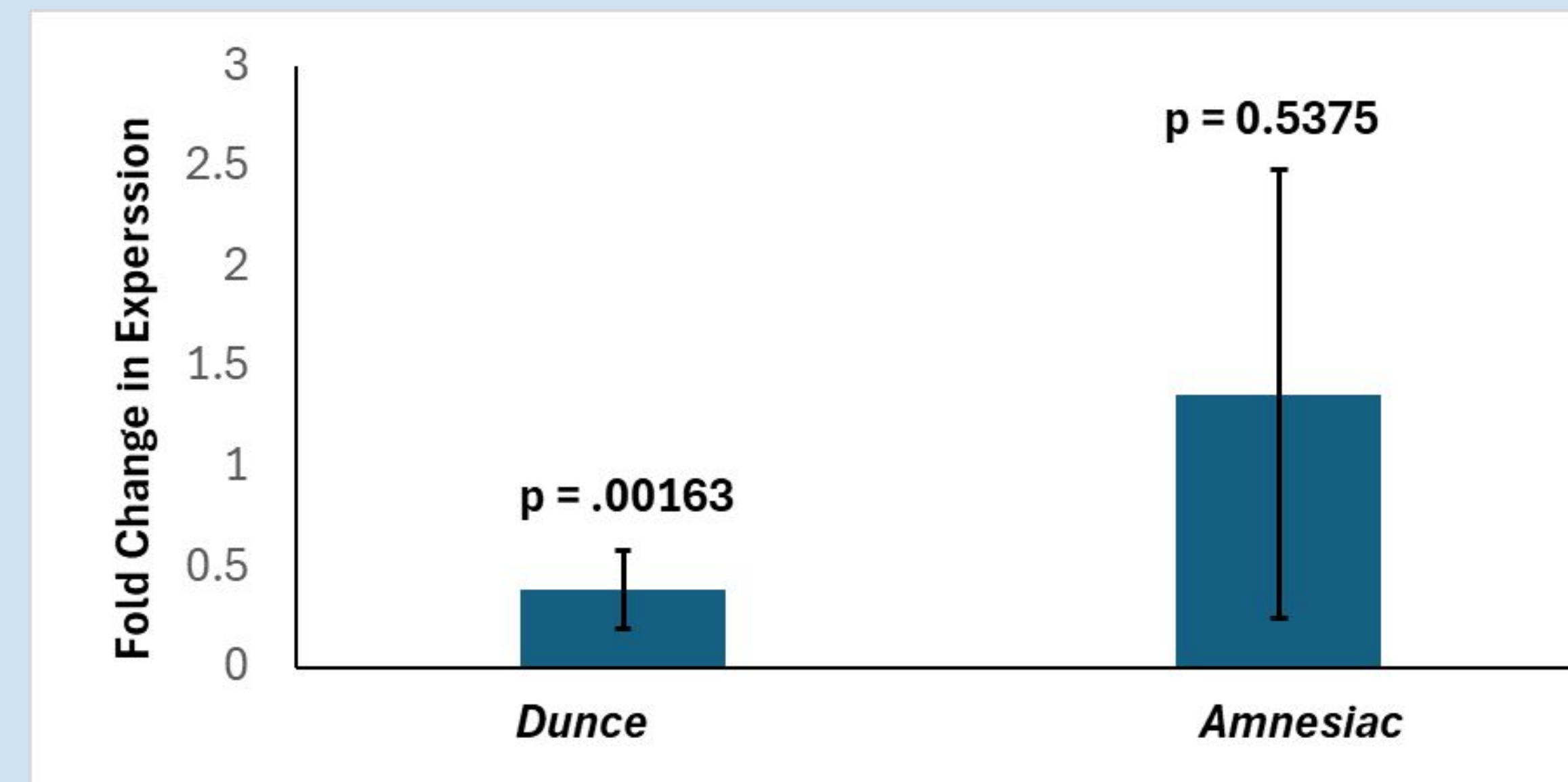
## Introduction

- Alpha-GPC is a supplement proposed to enhance cognitive ability and physical performance through the synthesis of Acetylcholine (ACh).
- Studies show that alpha-GPC can be beneficial in treating scopolamine-induced amnesia in rats, Alzheimer's, and other memory disorders
- With its increased popularity among people seeking cognitive enhancement, more research is needed to substantiate its claims
- *Dnc* and *amn* are two well-studied genes in the *D. melanogaster* brain that are crucial to memory-forming pathways
- **Hypothesis:** We hypothesized that alpha-GPC treatment in *Drosophila melanogaster* would increase expression of *amn* and *dnc*, as well as improve memory and locomotion as measured through behavioral assays.

## Methods

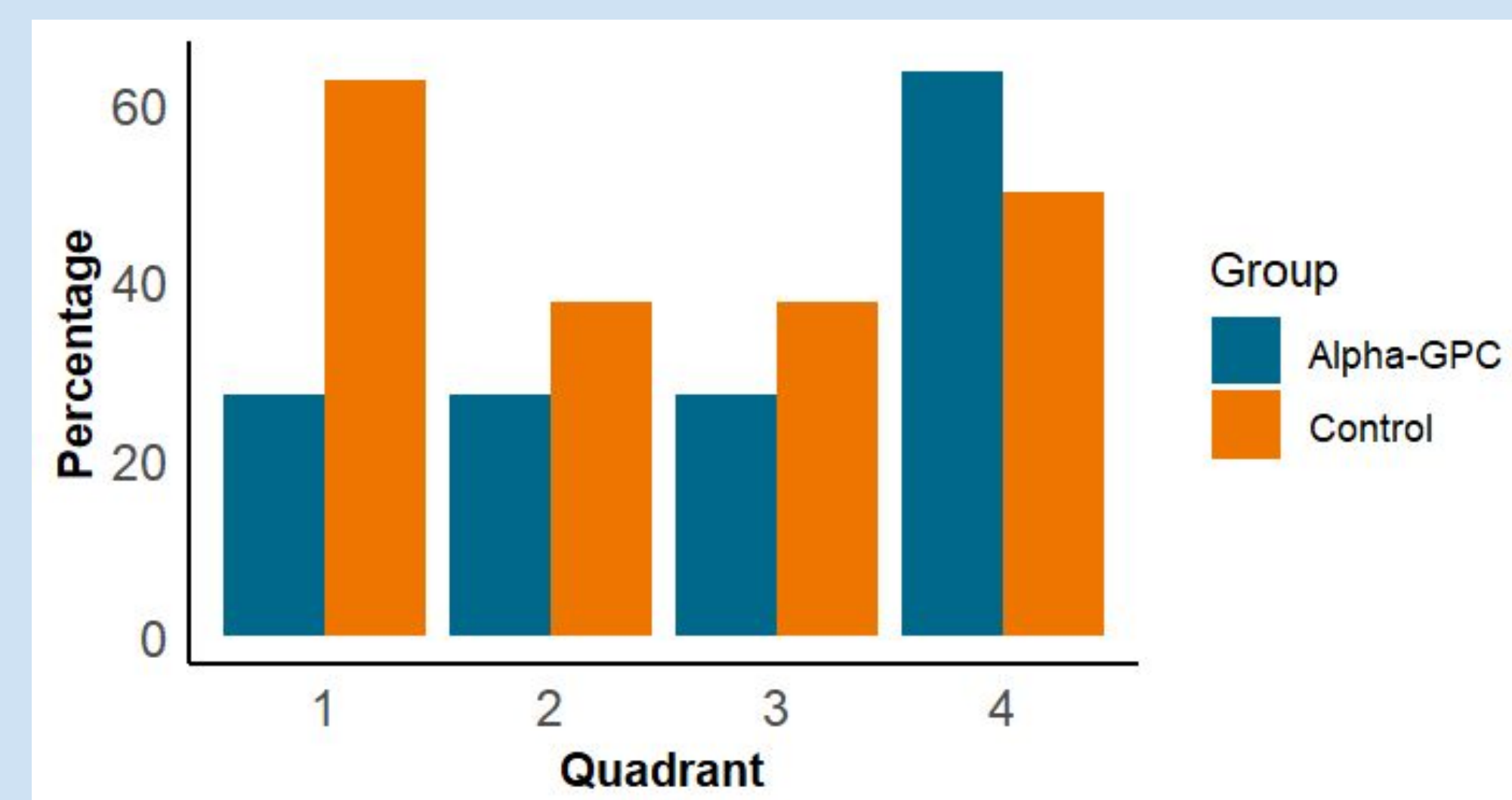
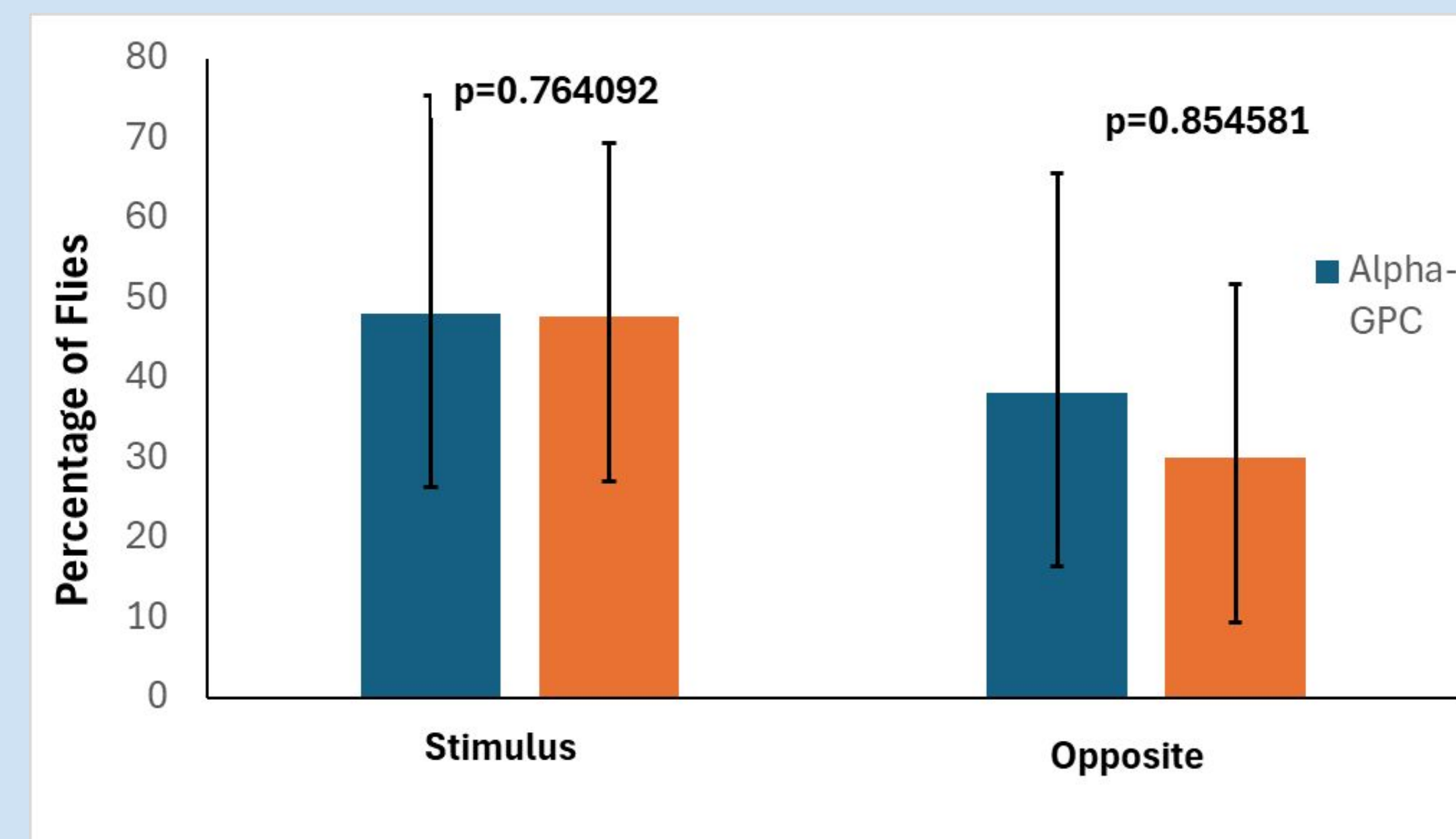
- **Fly Culturing:** *Drosophila melanogaster* were cultured in 20 mL vials with potato flakes, yeast pellets, and RO water. Experimental cultures were treated with alpha-GPC (600 mg in 10 mL RO water, 61.5 μL added per vial). Cultures were incubated at room temperature under a 12-hour light/dark cycle.
- **RNA Extraction:** RNA was extracted from L3 larvae using the Qiagen RNeasy Kit. Twenty larvae per group were homogenized in Buffer RLT+β-Me and purified with spin columns. RNA concentration was measured via NanoDrop and stored at -20°C until RT-qPCR.
- **RT-qPCR:** RNA was reverse-transcribed to cDNA using the RevertAid RT kit. Gene-specific primers for *dnc* and *amn* were used with Gapdh as a reference. qPCR was performed with iTaq SYBR Master Mix, and Ct values were used to compare gene expression.
- **Olfactory Memory Assay:** Flies were starved for 6 hours, conditioned with apple cider vinegar in vials covered with blue or green cellophane, and tested in a Y-maze with matching arms. Fly choices were recorded, and preferences analyzed using a two-sample T-test.
- **Phototaxis Assay:** Flies were placed in a 28 cm tube with a blue light source. Movement toward the light was recorded over 5 minutes. Six replicates were performed, with light preference analyzed via ANOVA analysis.
- **Wall Climbing Assay:** Flies in 9.5 cm vials were tapped to the bottom, and climbing behavior was recorded after 30 seconds. Trials were repeated three times per group, with six replicates total. Geotactic behavior was compared using a two-sample T-test.

## Results

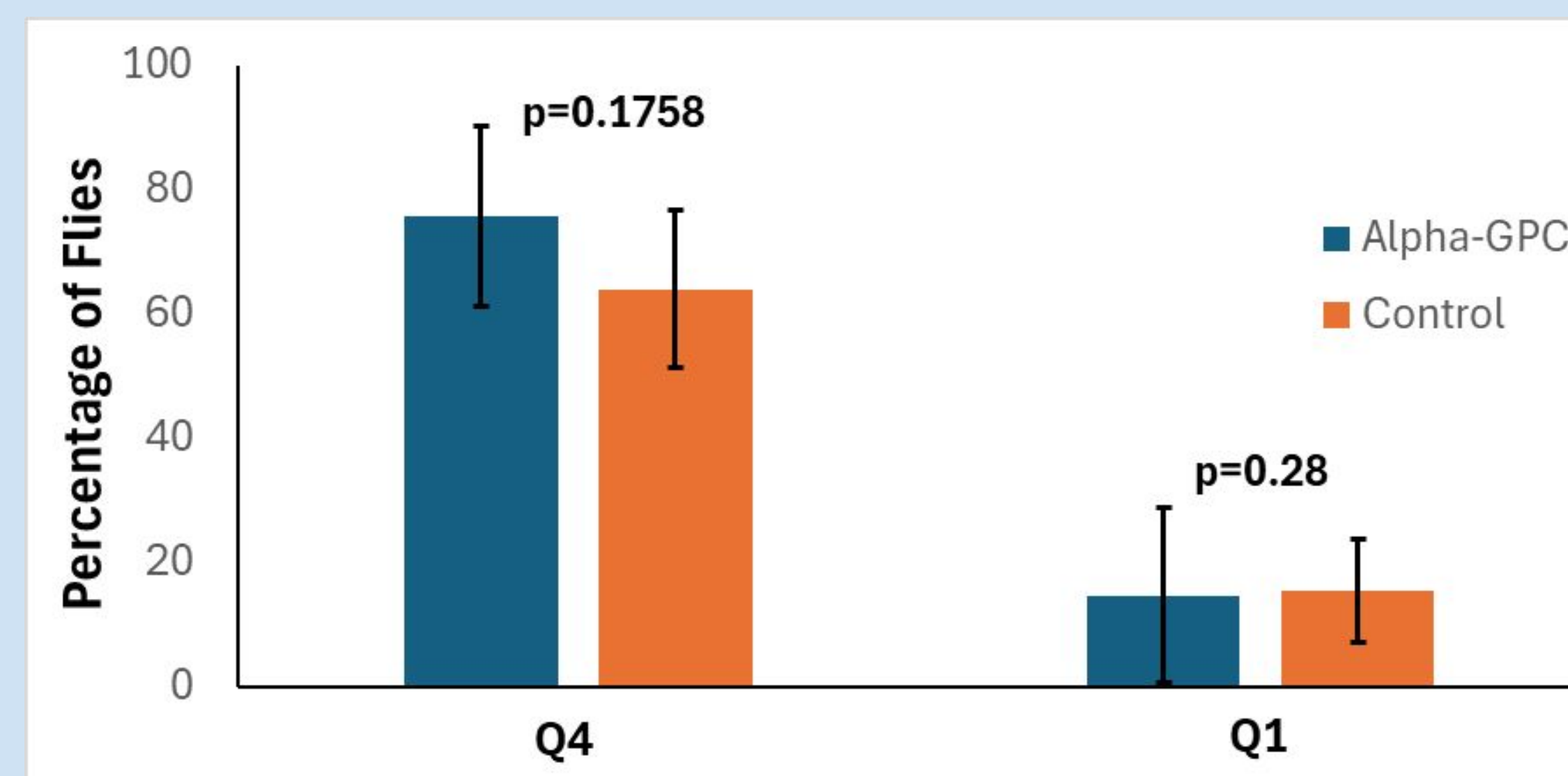


**Figure 1.** Average fold change in the expression of *dnc* and *amn* in flies exposed to alpha-GPC compared to control flies. (In *dnc*,  $p=0.00163$ ,  $n=4$ . In *amn*,  $p=0.5375$ ,  $n=5$ ). Error bars represent the standard deviation.

**Figure 2. Olfactory Memory Assay.** Stimulus represents the percentage of flies that chose the color associated with the attractive stimulant. Opposite represents the percentage of flies that chose the color that had no stimulant. Flies that were undecided are included in the sample number but not shown on the graph. (stimulus:  $p=0.764092$ , Opposite:  $p=0.854581$ , Control:  $n=172$ , Treatment:  $n=167$ )



**Figure 4. Geotaxis Assay.** Q4 represents the top quadrant closest to the stopper and Q1 represents the bottom quadrant. Percentage of flies in each group is shown on the y axis (Q4:  $p=0.1758$ , Q1:  $p=0.28$ ) (Control:  $n=444$ , Treatment,  $n=397$ )



**Figure 3. Phototaxis Assay.** ANOVA analysis of percentage of flies that traveled closest to the light (Q4) or stayed near the stopper (Q1). Flies were counted every minute for five minutes. ( $p=0.00771$ ,  $n=1469$ )

## Conclusions

- Five rounds of RT-qPCR showed no significant change in *amn* expression ( $p=0.5375$ ).
- Four rounds of RT-qPCR showed a significant change in *dnc* expression ( $p=0.00163$ ).
- Seven rounds of behavioral assays showed no significant difference in wall-climbing behavior. ( $p=0.1758$ ;  $p=0.28$ ) or Olfactory Memory behavior ( $p=0.764$ ;  $p=0.8546$ )
- A significant increase in phototaxis behavior ( $p=0.00771$ ) was observed over the seven rounds of behavioral assays.
- Future studies should focus on increasing the number of replicates for each assay, targeting other genes related to choline synthesis and degradation, as well as investigation into long-term memory behaviors.

## References

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