

# The Effects of Brain-770 on Sleep, Response Time, and Social Interaction in *Drosophila melanogaster*

Sarah Ries, Sorren Reese, and Dr. Otto-Hitt

Department of Biology, Carroll College

## Introduction

- *Drosophila melanogaster*, the common fruit fly, is a popular model organism in Neuroscience research.
- Prior research has found connections between increased pineal gland calcification and behaviors including response time, social interaction, and sleep.
- Pineal gland calcification plays a role in decreased melatonin production, contributing to conditions such as Alzheimer's Disease and Dementia.
- Brain-770 is a homeopathic supplement that is marketed as a treatment for pineal gland calcification.
- The *Cry1* gene encodes a photoreceptor protein that signals melatonin production when no light is detected.
- The *5-HT1B* gene encodes serotonin, which acts as a precursor to melatonin production.
- **Hypothesis:** It was hypothesized that Brain-770 would increase expression of *Cry1* and *5-HT1B* while also increasing sleep quality, social interaction, and response time.

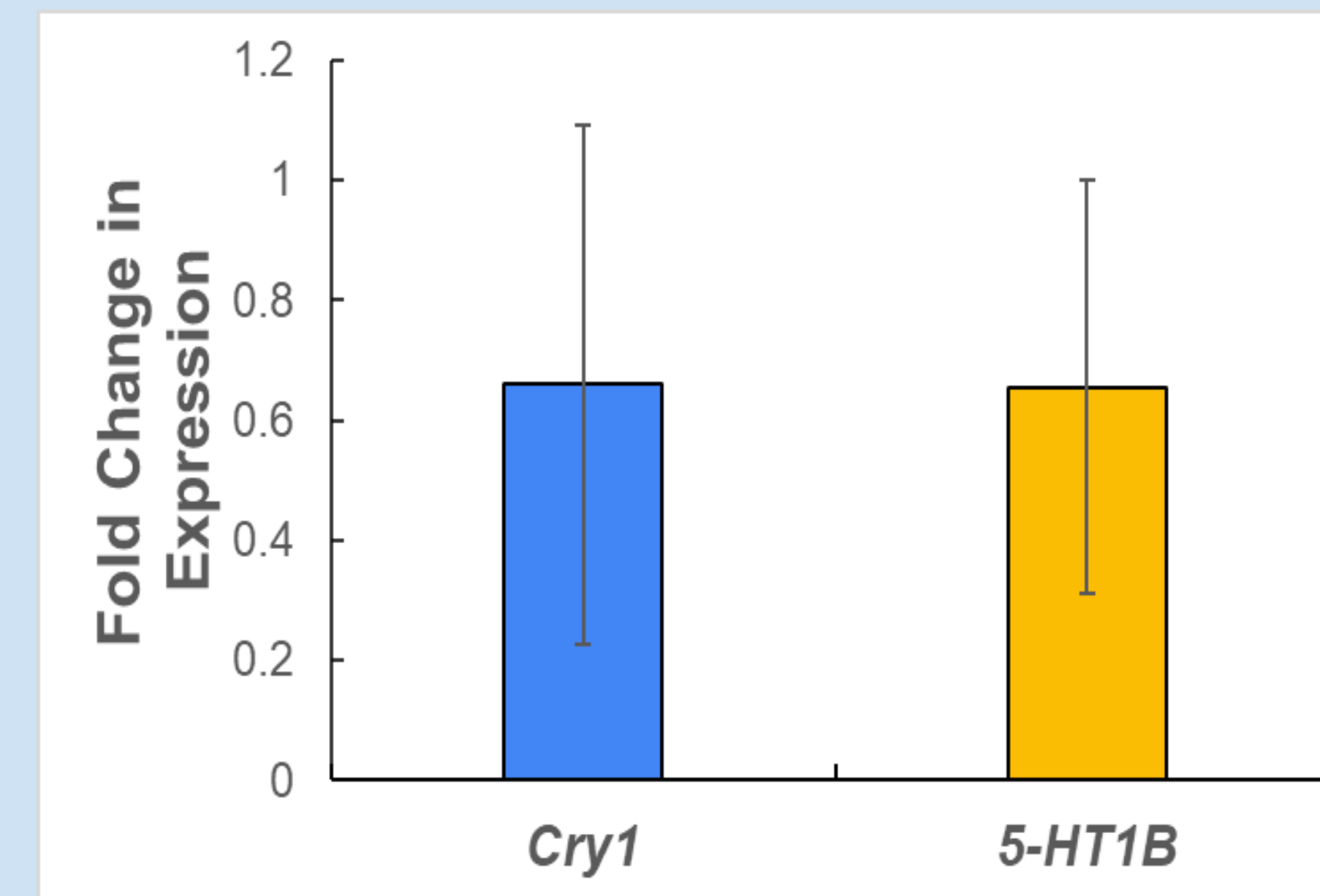
## Methods

- **Primer synthesis:** Primers for *Cry1* and *5-HT1B* were designed using NCBI primer design software.
- **Culturing:** *D. melanogaster* cultures were maintained in Instant *Drosophila* Medium. Experimental cultures were treated with 62 $\mu$ L of Brain-770 treatment solution and kept in a 20:4 hour light-dark incubator.
- **RNA extraction:** RNA was extracted using the Qiagen RNeasy extraction kit and procedure.
- **RT-qPCR:** Extracted RNA was subjected to reverse transcription using the RevertAid RT kit. PCR was performed using the iTaq SYBR Kit and *GAPDH* was used as a positive control.
- **Geotaxis Assay:** The number of *D. melanogaster* that climbed over 2cm in 10 seconds was counted.
- **Social Interaction Assay:** The number of interactions between *D. melanogaster* in one minute was counted.
- **Sleep Assay:** The number of *D. melanogaster* that fell asleep in 5 minutes was counted.

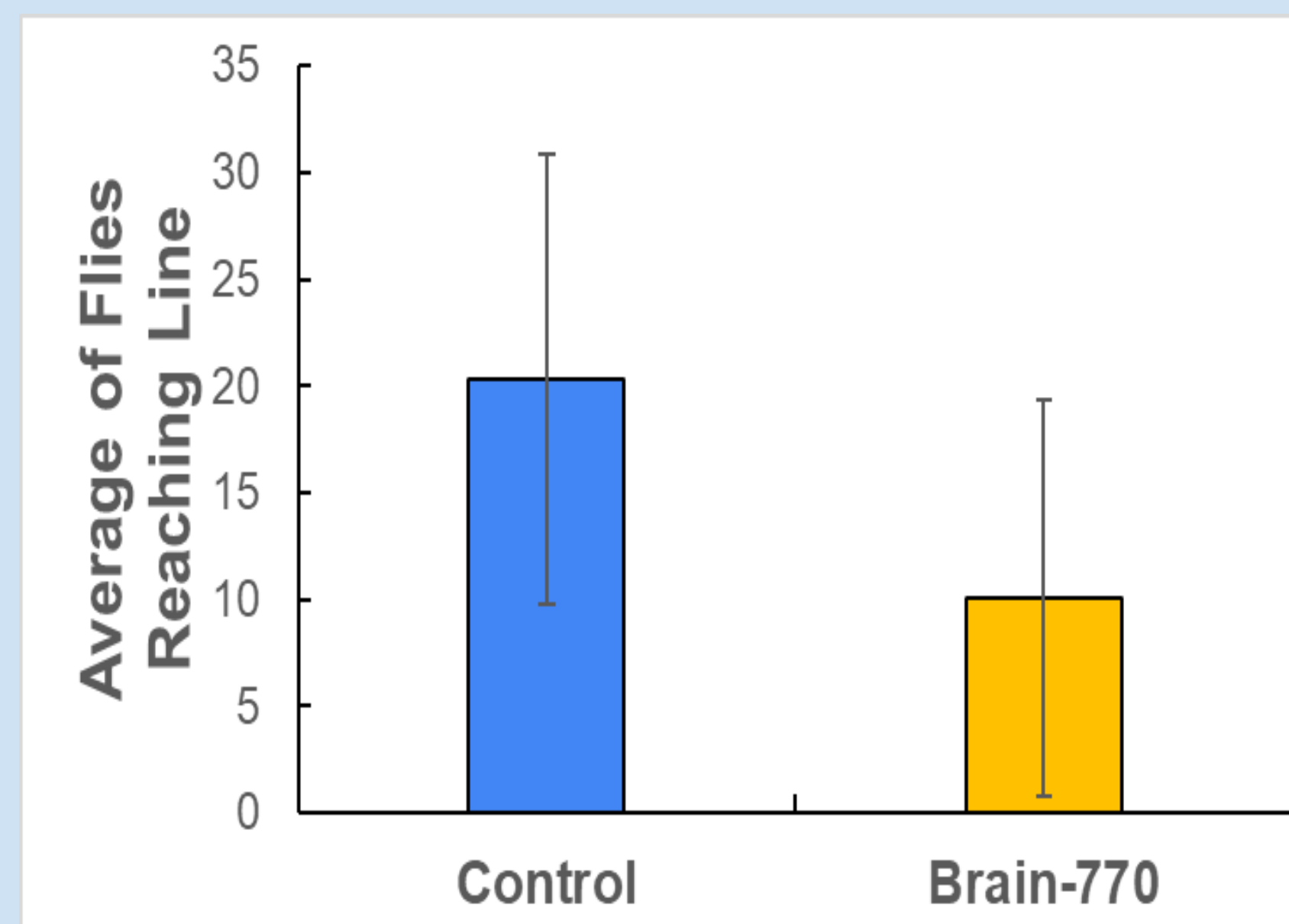
## Results



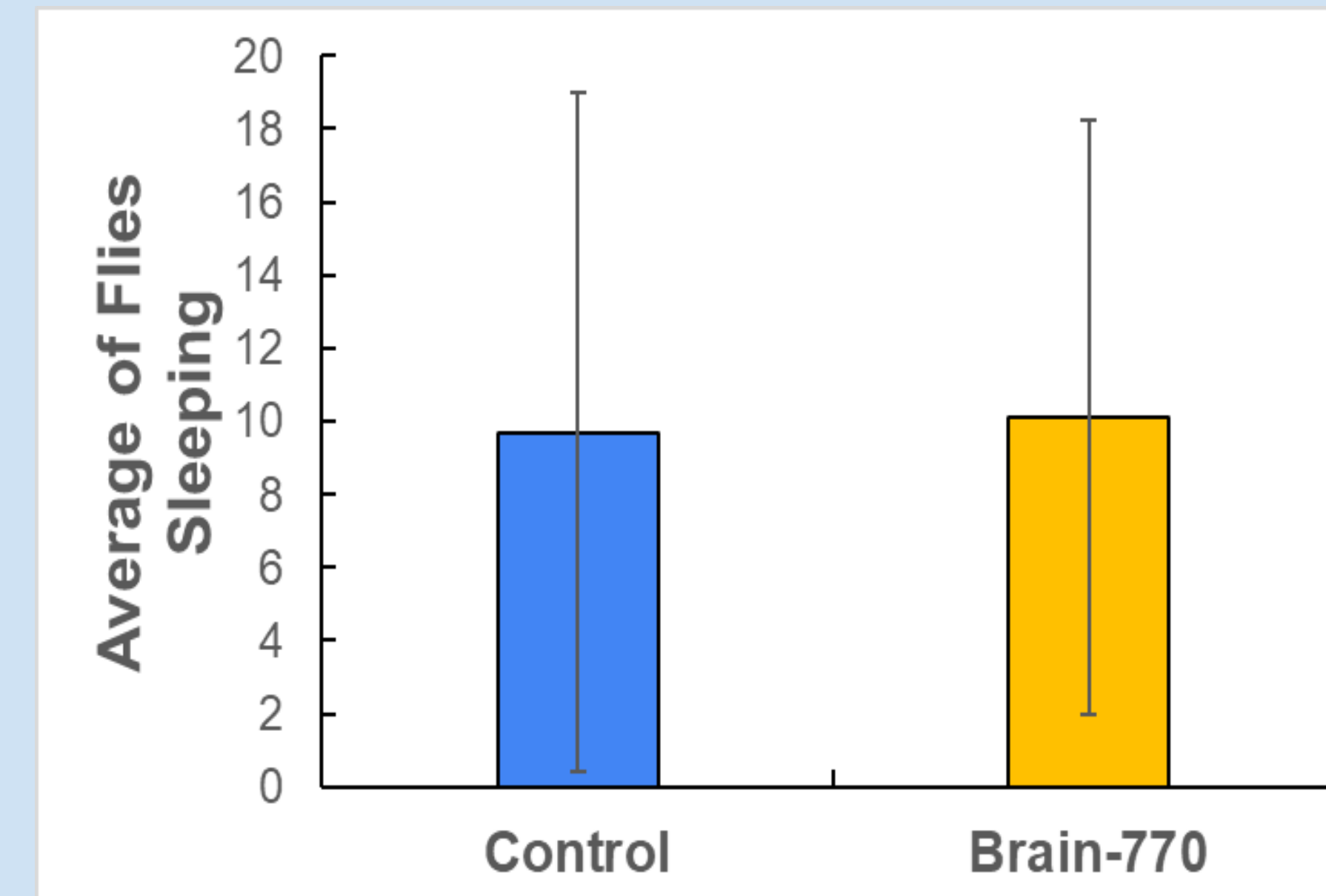
**Figure 1.** Brain-770 homeopathic supplement. Picture taken from top-probiotics.com.



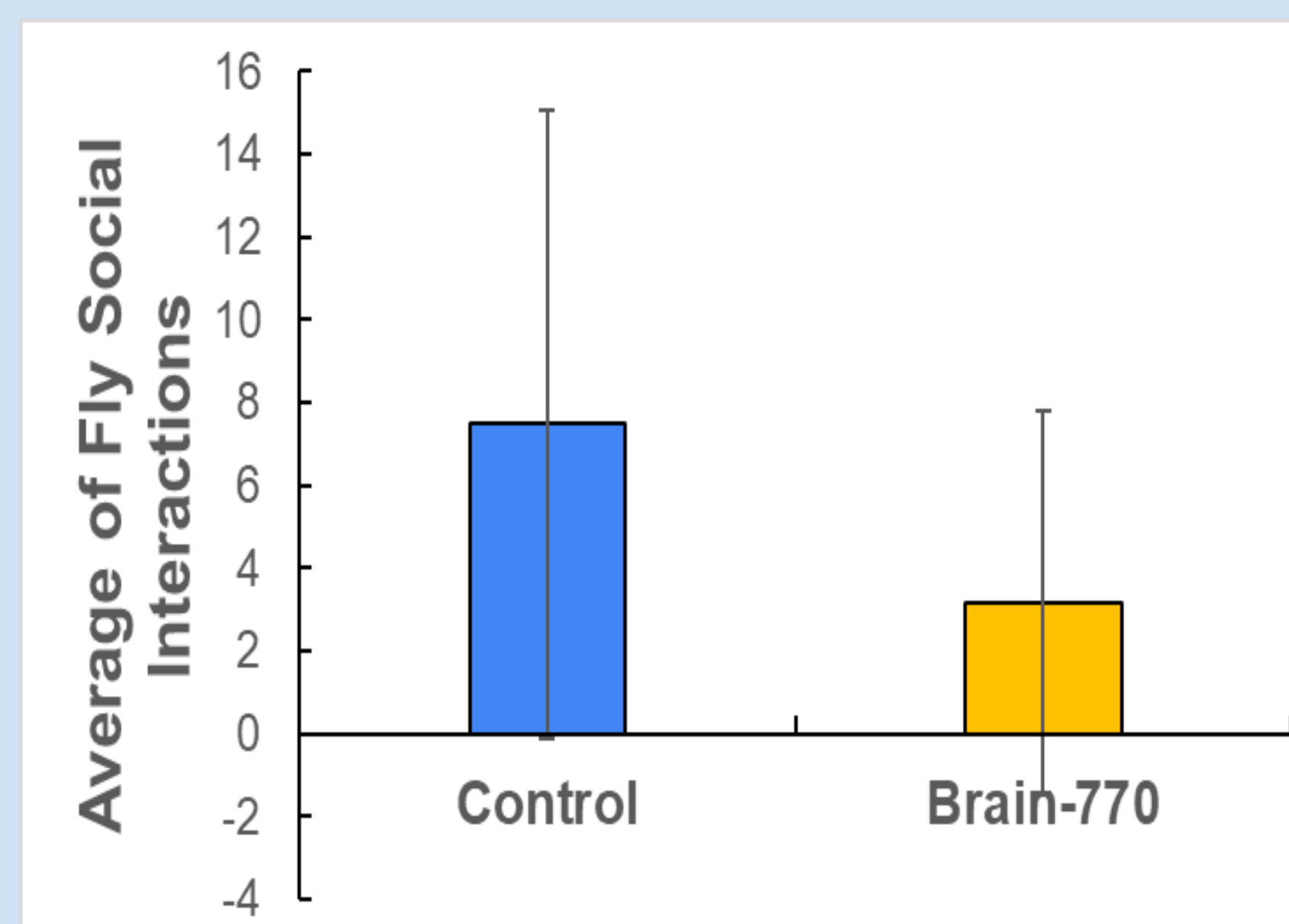
**Figure 2.** Fold change in expression of *Cry1* ( $p=0.152316$ ,  $n=6$ ) and *5-HT1B* ( $p=0.08880$ ,  $n=5$ ). Error bars represent standard deviation.



**Figure 3.** Results of geotaxis assays averaged across eight rounds. Error bars represent standard deviations ( $p=1.482E-5$ ,  $n=40$  for each group).



**Figure 4.** Results of sleep assays averaged across eight rounds. Error bars represent standard deviations ( $p=0.8882$ ,  $n=16$  for each group).



**Figure 5.** Results of social interaction assays averaged across eight rounds. Error bars represent standard deviations ( $p=0.003$ ,  $n=40$  for each group).



**Figure 6.** Social interactions assay of control *D. melanogaster*.

## Conclusions

- RT-qPCR results demonstrate that there was no significant difference in *Cry1* or *5-HT1B* expression between Brain-770-treated and control *D. melanogaster*.
- Behavioral assay results demonstrate that there was no significant difference in sleep, social interaction, or response time between treatment and control *D. melanogaster*.
- The results disagree with the hypothesis because no significant changes in gene expression or behavior were observed.
- Further research could include analysis of additional genes, additional behavioral assays, and more replicates.

## References

1. Bukreeva I, Junemann O, Cedola A, Brun F, Longo E, Tromba G, Wilde F, Chukalina M. V, Krivonosov Y. S, Dyachkova I. G, Buzmakov A. V, Zolotov D. A, Palermo F, Gigli G, Otlyga D. A, Saveliev S. V, Fratini M, & Asadchikov V. E. Micromorphology of pineal gland calcification in age-related neurodegenerative diseases. *Medical physics*. 2022 October 17;50(3), 1601–1613.
2. Chen C, Tamai TK, Xu M, et al. Functional Analyses of Four Cryptochromes From Aquatic Organisms After Heterologous Expression in *Drosophila melanogaster* Circadian Clock Cells. *J Biological Rhythms*. 2024 March 28;39(4):365-378.
3. El Maataoui Z, Belhadga H, Kisra H. Calcification of the Pineal Gland and Psychopathology in Obsessive Compulsive Disorder in Children and Adolescents. *Sch Acad J of Biosci*. 2023 Oct 24;11(10):371-374.
4. Hyun Lee B, Hille B, Koh DS. Serotonin modulates melatonin synthesis as an autocrine neurotransmitter in the pineal gland. *PNAS*. 2021 Oct 21;118(43)5.
5. Wade A. G, Farmer M, Harari G, Fund N, Laudon M, Nir T, Frydman-Marom A, & N. Add-on prolonged-release melatonin for cognitive function and sleep in mild to moderate Alzheimer's disease: a 6-month, randomized, placebo-controlled, multicenter trial. *Clin interv aging*. 2014 June 18;9, 947–961.

## Acknowledgements

We would like to thank Dr. Otto-Hitt for her assistance and guidance throughout the research process.