The Effect of Optogenetic Modulation of Serotonin Neurons on the Behavioral States of Restrained *Drosophila Melanogaster*

Izabelle Kokkat[1,2] , Haomeng Li[2], Ada Okoluku[2], Kyle Gobrogge[2]

Notre Dame High School, San Jose, CA 95112, USA[1] ; Undergraduate Program in Neuroscience, Boston University, Boston, MA, USA[2]

Introduction

- *Drosophila melanogaster* (fruit flies) have evolved their defense mechanisms to enter two distinct behavioral states when restrained: **periods of flailing (active) and periods of immobility (inactive)**
- Serotonin (5-HT) is known to **influence responses related to stress, anxiety, and fear** and **enhance behavior inhibition**
- Studies conducted have found **significant sex-differences** in how the fruit flies respond when being restrained
- **Expected results based on similar studies**
  - Activation of 5-HT neurotransmitters would yield a lower activity-inactivity ratio and less switching between states
  - Female flies would have a higher activity-inactivity ratio and more switching between states
- Using **optogenetics**, we can use red and yellow light to activate or inactivate targeted neurotransmitters and study how this affects the frequency and duration of the activity and inactivity periods
  - This works by using genetic crossing to express **the existing voltage gated 5-HT ion channels as light gated channels** in the progeny
- *Drosophila* have been proven as an accurate model for understanding complex human conditions such as PTSD
  - Understanding the role of 5-HT in stress and trauma responses in Drosophila can inform the development of **more effective therapeutic strategies for PTSD and related disorders**

References/Supplemental Materials

Results

A

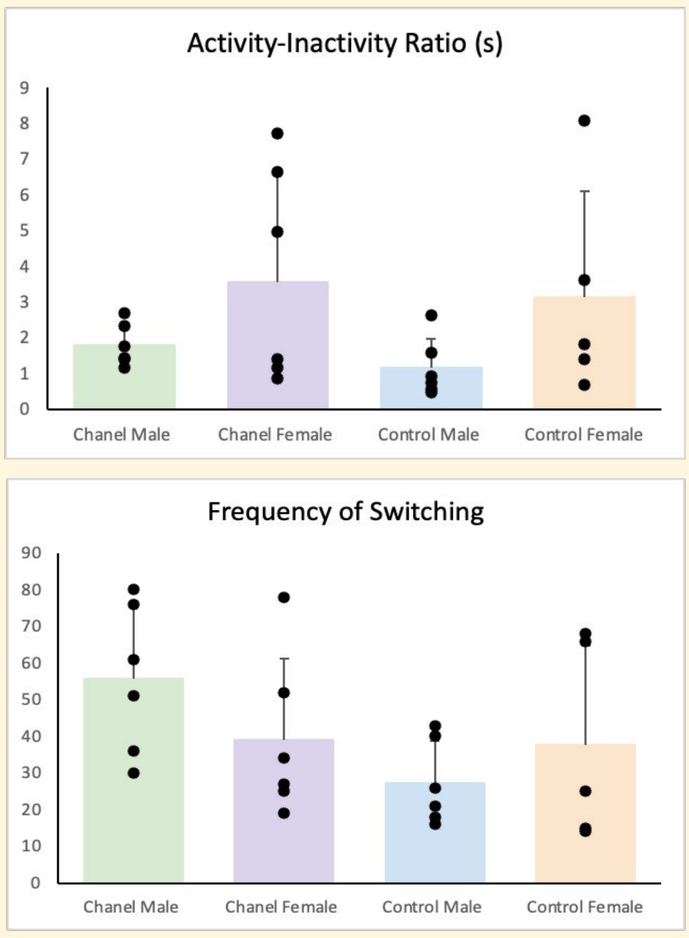
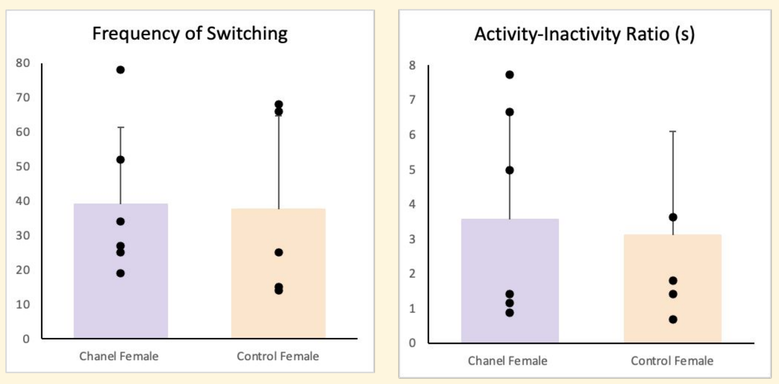


Fig.3 Acute Activation Male and Female Groups and Control Groups: A) Activity-Inactivity Ratios (found by dividing time spent active by time spent immobile). B) Frequency of Switching (number of distinct intervals of activity or inactivity the fly entered)

A



B

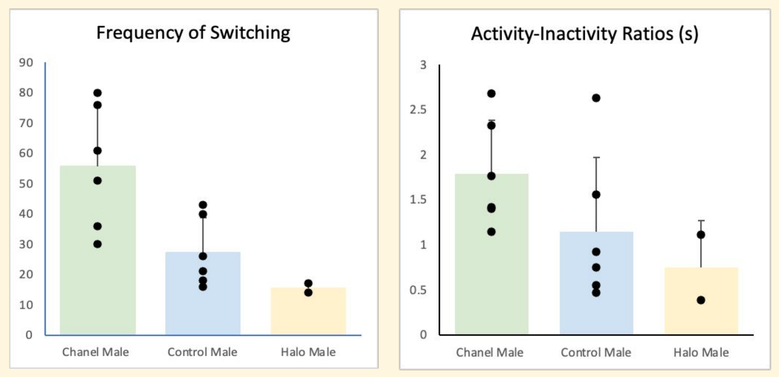


Fig.4 Male and Female Fly Groups: A) Female experimental and control groups B) Male experimental and control groups

Discussions

- In both male and female fly groups, **acute activation** of 5-HT neurons resulted in **higher average activity-inactivity ratios and more switching between states**
- In male flies, **acute inactivation** of 5-HT neurons resulted in **lower average activity-inactivity ratios** and **less switching between states**
  - Although not the expected results, these outcomes suggest that serotonin modulation does have some impact on the behavior of the restrained flys
- **Female fly groups** have **significantly higher average activity-inactivity ratios** than male fly groups
  - This outcome could inform consideration of sex as a factor in future studies about human stress responses
- There is **no clear association** between sex and the **frequency of switching between states**
- **Possible limitations:** limited sample size, variability of testing conditions
- In conclusion, this study demonstrates the possibility of a correlation between serotonin modulation and behavioral states of restrained fruit flies
- Additional testing could explore the effect of long term serotonin modulation on these areas of behavior and how this can be applied to human stress responses and possible treatment methods

Acknowledgements

I would like to thank Dr. Kyle Gobrogge and the mentors for guiding me through this process and showing me the possibilities of the GAL4-UAS system. I would also like to thank the RISE program for giving me this opportunity.

Methods

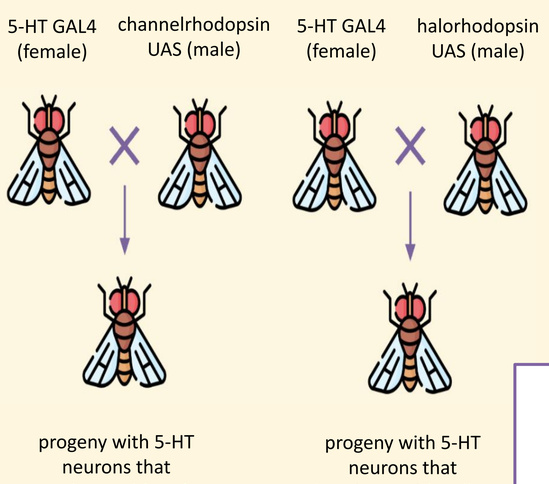


Fig.1 Genetic Crosses: Utilizing the GAL4-UAS system and optogenetics, we can use yellow and red light frequencies to selectively activate or deactivate 5-HT neurons in the progenys

Image.1 Immobile Fly: Depicts male chanel fly immobilized on slide

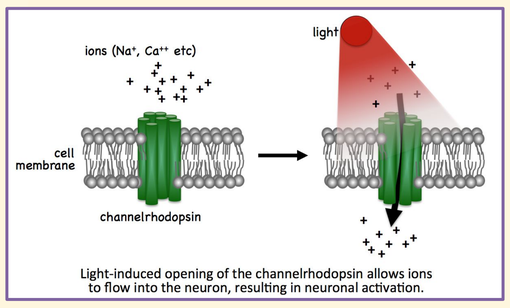


Fig.2 Effect of Light on Ion Channels of the Progeny: Depicts light gated Na+ ion channels

Restrained Behavioral States Assay

- Each trial involved using clear nail polish to immobilize the fly on a slide so that it could move its abdomen and legs and recording its behavior for 5-14 minutes
- Periods of activity consisted of the fly thrusting its abdomen and kicking its legs
- Red or yellow light was shined on the flies for the duration of the trial
- 5-minute excerpts of the videos were analyzed by hand to determine the frequency at which the flies switched between states and the duration

Optogenetics

- Channelrhodopsin couples with the ion channels in 5-HT neurons and allows red light to open the channel, depolarizing the neuron and activating it
- A similar process happens with halorhodopsin and yellow light, instead hyperpolarizing the neuron and deactivating it

Visualization

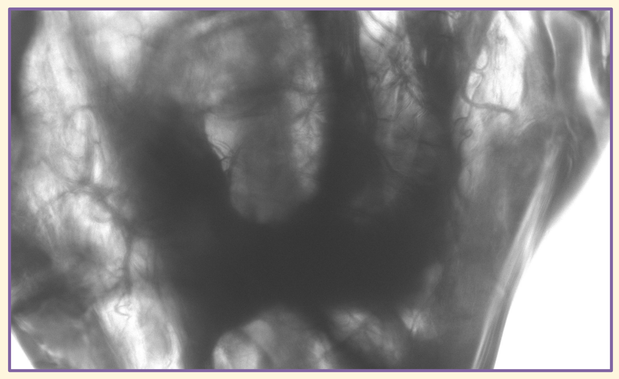


Image.2 Larvae Brain Under Leica Microscope

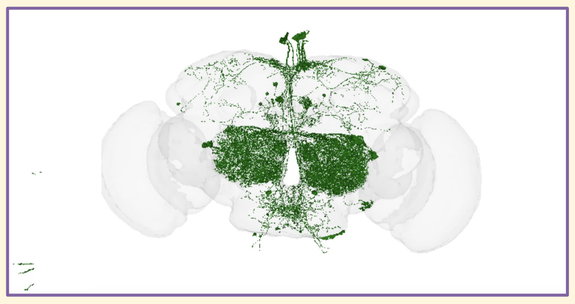


Image.3 Random Sample of 100 5-HT Neurons in FlyWire AI