Optogenetic Modulation of Serotonin Neurons Reveals Sex-Specific Differences in Negative Geotaxis in Drosophila: A Model for Measuring Depression-Like Behavior

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Introduction

- Depression is a mental disorder affecting millions worldwide.
- Serotonin is a neurotransmitter known to control mood, perception, anger, and more.
- A common type of antidepressant known as serotonin reuptake inhibitors (SSRIs) works by increasing serotonin levels in the brain.
- The partial success of these drugs led to the serotonin theory of depression, which proposes that a chemical imbalance of serotonin drives depression.
- Despite emerging evidence challenging this theory, it has not been definitively disproved.
- Depression is also more prevalent in women than in men, but the underlying causes of this disparity remain unclear.
- In this study, we utilized optogenetics to activate and inactivate serotonergic neurons in the *Drosophila melanogaster* model to measure the changes in sex-specific responses to induced depression.
- The objectives of this study are to explore the role of serotonin in depression and investigate the causes driving the differences in depression between sexes.

Materials & Methods

We used the GAL4-UAS system to create a fly cross with activatable/deactivatable serotonergic neurons.

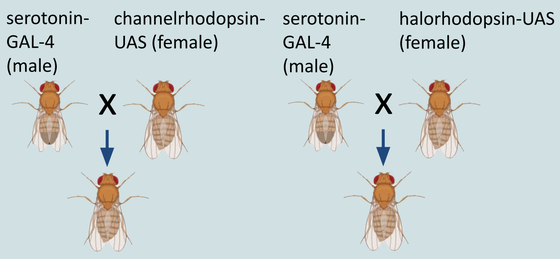
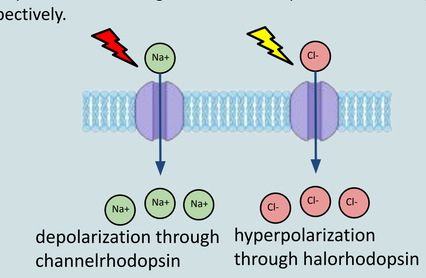


Fig. 1: Genetic crosses
The progeny contain a GAL4 transcriptional activator regulated by a promoter typically expressed in serotonin neurons, and a UAS enhancer activating promoters for channelrhodopsin and halorhodopsin ion channels. These proteins depolarize and hyperpolarize serotonergic neurons in response to red and yellow light respectively.



Images created with Biorender.com

Fig. 2: Effect of light on ion channels of progeny

A forced climbing assay was used to exploit Drosophila's innate negative geotaxis behavior.

- The assay device was created by connecting two 9.4 cm tall vials, and was then split into four even quadrants which were marked with Sharpie.
- Male/female progeny were placed in separate devices.
- Climbing activity was collected for three hours across six 30-minute periods each containing 30 one-minute-long trials to simulate learned helplessness. The movement impairment measured across the three hours is analogous to features of depression like decreased activity and anhedonia.
- Negative geotaxis is stimulated by slamming flies down for five seconds. Flies were given 25 seconds to climb, and data was collected in the following 30 seconds.
- Red and yellow light was shown on experimental groups throughout the assay duration to activate and deactivate neurons respectively. All tests were done between 6:30 pm and 10 pm.
- The average quadrant flies ended up in was collected after every trial. A higher quadrant crossed into represented greater climbing activity.

Results

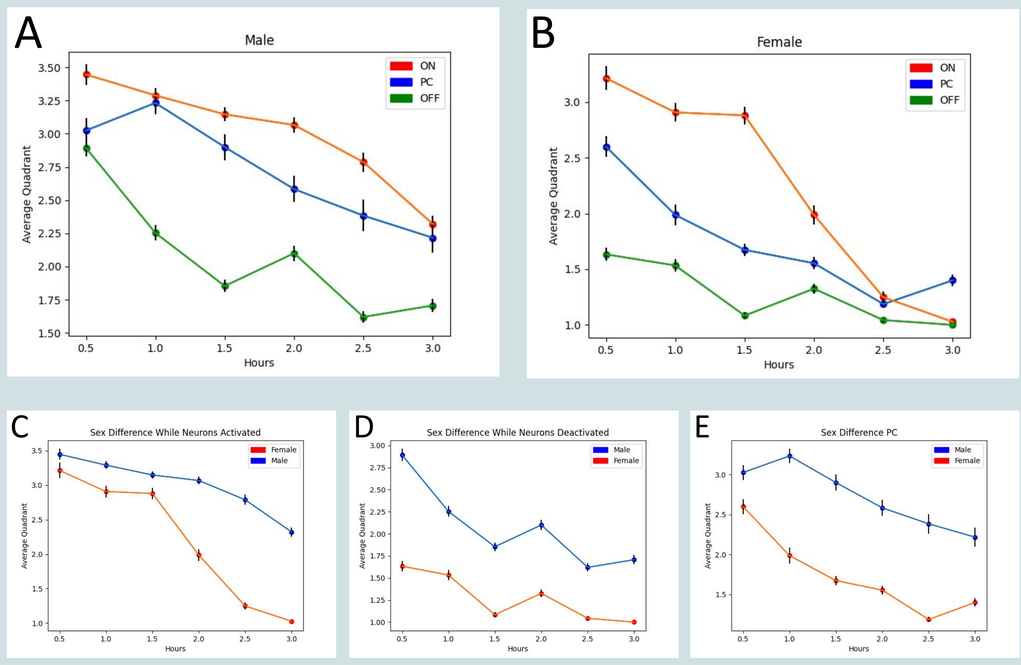
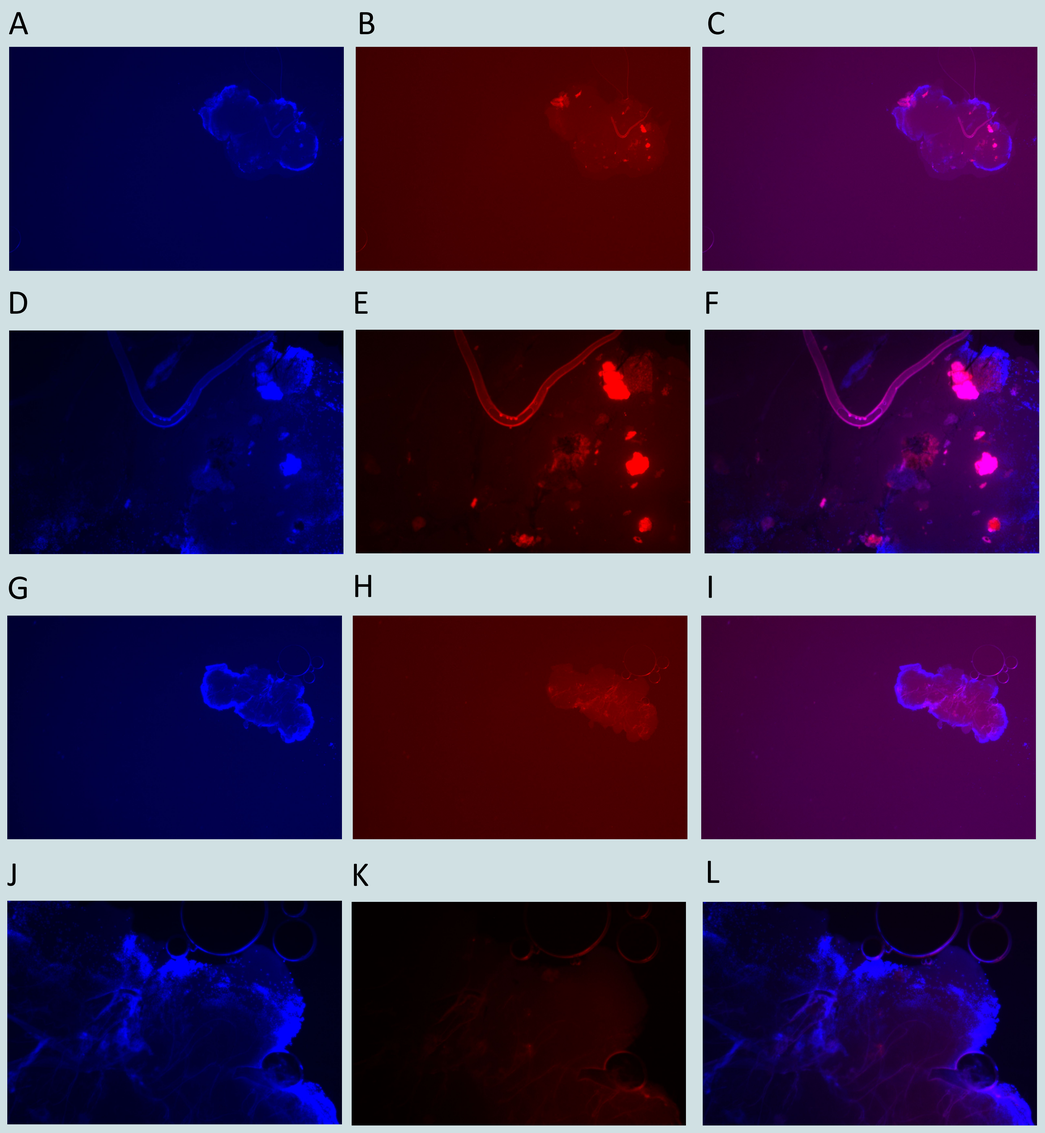


Fig. 3: Effect of serotonin modulation climbing activity (mean ± SEM) A) Effect of the assay on male flies across all levels of serotonin modulation B) Effect of the assay on female flies across all levels of serotonin modulation C) Sex-specific climbing differences while neurons activated D) Sex-specific climbing differences while neurons inactivated E) Sex-specific climbing differences with parental control. Data analyzed using Mixed Three-way Repeated Measures Anova. Effect of serotonin treatment $p < 0.0001$; effect of sex $p < 0.0001$; interaction of treatment and sex $p < 0.003$; effect of time $p < 0.0001$; interaction of time and treatment $p < 0.0001$; interaction of time and sex; $p < 0.0001$; interaction of treatment, sex, time $p < 0.0001$

Visualization



Fluorescent microscopy imaging was taken as proof of work for the fly crosses. Images processed with ImageJ. Glowing pink areas in merged images are serotonergic neurons. (C, F, I, L). Red images depict channelrhodopsin/halorhodopsin fluorescent protein tags, blue images depict DAPI counterstain.
(A) 4x DAPI, (B) 4x 5-HT-ChR, (C) 4x merge.
(D) 20x DAPI, (E) 20x 5-HT-ChR, (F) 20x merge.
(G) 4x DAPI, (H) 4x 5-HT-NpHR, (I) 4x merge.
(J) 20x DAPI, (K) 20x 5-HT-NpHR, (L) 20x merge.

Conclusions

- Results show significant effects of the negative geotaxis assay as climbing activity drops over time in all categories, which may be a result of learned helplessness.
- Acute activation of serotonin neurons increases climbing activity while deactivation decreases activity.
- These results may be caused by interaction between 5-HT receptors and activation of adjacent sodium ion channels involved in motor neurons.
- The data supports the serotonin theory of depression, as increasing serotonin levels alleviate the depressive phenotype induced by the assay.
- Female flies are shown to be affected more by the induced depression than males (Fig. 3 C, D, E).
- This effect may be caused by differences in serotonin levels between the sexes, which may increase effects of anhedonia and movement impairment.
- The data demonstrates a chemical basis for the difference in depression-like effects between the sexes.

Future Directions

- Redo the experiment with automated Trikinetics Vortexer Mounting Plate and Drosophila Activity Monitor technology for greater accuracy.
- Utilize greater sample sizes to capture more variability.
- Do a general activity test with the same subjects to ensure changes from optogenetic modulation in the forced-climbing assay are climbing-specific.
- Apply the *Drosophila melanogaster* optogenetics model to other model organisms and eventually humans to study the drivers behind depression and test new therapeutic treatments.

References

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