

Anhedonic Behavior Resulting From Serotonin Depletion in *Drosophila*

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INTRODUCTION

Depression is a psychological disorder characterized by loss of interest and pleasure in activities, problems with sleeping, eating, energy, concentration, and self worth.¹ In 2021, it is estimated that 21 million adults had at least one depressive episode.² Depressive episodes are associated with increased mortality rates.³ Despite the abundance of serotonin research, the rate of depression, a fatal psychological disorder, is still quickly rising.⁴ Therefore, the effects of serotonin depletion on behavior needs further research. Serotonin is a neurotransmitter that is crucial in managing mood, reward, and motivation. In studies involving animal models, a lack of serotonin is frequently linked to behaviors resembling depressive-like symptoms observed in humans.⁴ Previously, serotonin research focused on *drosophila* larvae, however, our research will add to the literature by studying adult female social, feeding, and locomotive behavior.⁵

Previous literature has established a strong correlation between serotonin and depression. We will use *Drosophila* as an animal model in order to better understand the relationship between serotonin and depression by optogenetically depleting serotonin in female *Drosophila* to record their physiological and social behavior. By manipulating serotonin release in the *Drosophila* brain, their activity would mimic human depression. The specific behaviors we will be observing in adult flies are locomotion, fighting behavior, and food consumption.

We hypothesize that flies without proper serotonin release will exhibit overall reduced activity in movement, fighting behavior frequency, and food consumption. The serotonin neurons will undergo induced acute inactivation through isolated yellow light exposure for 1 hour. This inactivation will influence the behaviors in the experimental group. As the experimental and control group are genetically identical, causal effects of change in behavior can be isolated to serotonin depletion. Behaviors will be different from the control group—lacking yellow light exposure—which will indicate that observed effects are solely due to optogenetically induced depression. We have created two different *drosophila* cross lines. Our first cross is Gal 4 38389 (Chromosome 3) with UAS 25774 (Chromosome 2), which will be used to express mCherry and stain the serotonin neurons red for visualizing. Our second cross is Gal4 38389 (Chromosome 2)

¹ Major Depression. (July 2023). NIH. <https://www.nimh.nih.gov/health/statistics/major-depression>

² Ibid.

³ Patel, R., Arisoyin, A. E., Okoronkwo, O. U., Aruoture, S., Okobi, O. E., Nwankwo, M., Okobi, E., Okobi, F., & Momodu, O. E. (2023). Trends and Factors Associated With the Mortality Rate of Depressive Episodes: An Analysis of the CDC Wide-Ranging Online Data for Epidemiological Research (WONDER) Database. *Cureus*, 15(7), e41627. PMID: 37565131 PMCID: PMC10410604

⁴ Huser, Annina et al. "Anatomy and behavioral function of serotonin receptors in *Drosophila melanogaster* larvae." *PLoS one* vol. 12,8 e0181865. 4 Aug. 2017.

⁵ Sun Y, Qiu R, Li X, Cheng Y, Gao S, Kong F, Liu L, Zhu Y. Social Attraction in *Drosophila* is Regulated by the Mushroom Body and Serotonergic System. *Nature Communications*, 2020, 11(1): p 5350. PMID: 33093442.

with UAS 41753 (Chromosome 3), which will be used to express halorhodopsin in serotonin neurons. This will be a model of depression, when serotonin depletion is induced by exposure to yellow light to inactivate serotonin neurons selectively. We will investigate the impact of the serotonin depletion on specific behaviors related to depression symptoms.

HEALTH RELEVANCE STATEMENT

Drosophila melanogaster shares homologs with approximately 62% of human disease-causing genes and has been observed to have similar serotonin modulation to humans for sensory processes like olfaction, hearing, and vision.⁶ *Drosophila* has long been utilized as a model organism for depression⁷, which can be applied to models of human mental health.

To better understand how depression influences behavior, our research investigates how depressive symptoms develop by depleting serotonin and observing subsequent behavioral changes. This study aims to enhance understanding of how neurochemical alterations affect emotional regulation, possibly leading to the improvement of therapeutic strategies.

We are looking at the mechanism in which depressive symptoms emerge. We are acutely depleting serotonin to model depression and studying the resulting behavior to understand the effects of serotonin on depressive symptoms. Our research studying multiple behaviors in a single group of flies will contribute to knowledge of the disease: how serotonin depletion affects behavior and ultimately depression.

⁶ Fortini ME, Skupski MP, Boguski MS, Hariharan IK. A survey of human disease gene counterparts in the *Drosophila* genome. *J Cell Biol*, 2000. 150(2):F23-30. PMID: 10908582.

⁷ Sampson MM, Myers Gschweng KM, Hardcastle BJ, Bonanno SL, Sizemore TR, Arnold RC, Gao F, Dacks AM, Frye MA, Krantz DE. Serotonergic modulation of visual neurons in *Drosophila melanogaster*. *PLoS Genet*. 2020 Aug 31;16(8):e1009003. PMID: 32866139.

SPECIFIC AIMS

The serotonergic neurons broadly innervate the optic ganglia of *Drosophila Melanogaster* and it is a widely used model for studying vision. *Drosophila* has approximately 80 serotonergic neurons.⁸ Halorhodopsin is a chloride channel that hyperpolarizes the cell in response to yellow light. Utilizing a Gal4-UAS system, we created a cross of *Drosophila* that expresses halorhodopsin in serotonergic neurons. In recent studies, *Drosophila* has been shown to be a useful model system for researching depression.⁹ Serotonin is a neurotransmitter that is crucial in managing mood, reward, and motivation. In studies involving animal models, a lack of serotonin is frequently linked to behaviors resembling depressive-like symptoms observed in humans.⁴

While chronic serotonin deficiency has been studied, the immediate effects of acute serotonin disruption requires further research. Existing research often focuses on chronic manipulations of serotonin levels or genetic mutations, which may not fully explain the acute effects of serotonin signaling on behavior.¹⁰ The correlation between an acute depletion in serotonin and behaviors like feeding, fighting, and movement remains unclear. By measuring multiple behavior outputs, we aim to establish an association between acute serotonin disruption and anhedonic behaviors in female *Drosophila*. We hypothesize that a one hour of serotonin signal disruption will induce anhedonic behaviors in *Drosophila*, proven by reduced food consumption, decreased fighting frequency, and decreased locomotion. To begin studying this acute serotonin disruption, we will create an anhedonic fly, as demonstrated in these three behaviors: inhibited food intake, fighting, and movement.

Aims:

1. Our first aim is to create our transgenic cross using the Gal 4 and UAS systems. We will create two different *drosophila* lines: Gal4 38389 with UAS 25774 and Gal4 38389 with UAS 41753. Gal4 38389 with UAS 25774 will be used to express mCherry in the serotonin neurons to allow for visualization—the serotonin neurons will fluoresce red. Gal4 38389 with UAS 41753 will result in halorhodopsin expression in these serotonin neurons to allow for optogenetic inactivation of the serotonin neurons.
2. Our second aim is to image the brain, as proof of principle that we have successfully created our crosses. We will dissect the brains and use fluorescence microscopy to identify the fluorescing red serotonin neurons.

⁸ Kasture AS, Hummel T, Sucic S, Freissmuth M. Big Lessons from Tiny Flies: *Drosophila melanogaster* as a Model to Explore Dysfunction of Dopaminergic and Serotonergic Neurotransmitter Systems. *Int J Mol Sci*. 2018 Jun 16;19(6):1788. PMID: 29914172.

⁹ Ries AS, Hermanns T, Poeck B, Strauss R. Serotonin modulates a depression-like state in *Drosophila* responsive to lithium treatment. *Nat Commun*. 2017 Jun 6;8:15738. PMID: 28585544.

¹⁰ Bacqué-Cazenave J, Bharatiya R, Barrière G, Delbecque JP, Bouguiyou N, Di Giovanni G, Cattaert D, De Deurwaerdère P. Serotonin in Animal Cognition and Behavior. *Int J Mol Sci*. 2020 Feb 28;21(5):1649. PMID: 32121267.

3. Lastly, our third aim is to measure their behavior: locomotion, feeding, and fighting. We will perform DAM on the halorhodopsin cross in order to track their movement. We will remove the abdomens of the *Drosophila* and pass light through them, using spectrophotometry to quantify how much food was consumed based on light transmission. This will allow us to determine the eating behavior of the *Drosophila*. Lastly, we will set up a fighting chamber and record a video to high throughput their different behaviors—6 flies per trial, two groups: control and experimental.

RESEARCH STRATEGY

Significance: *Drosophila* Model of Social behavior

Understanding the neurobiological mechanisms that regulate behavior in response to neurotransmitter signaling is a fundamental challenge in neuroscience, particularly in studies of the serotonergic role in modulating aggression, feeding, and locomotive behaviors. Serotonin is a neurotransmitter that affects a wide array of behaviors in both humans and animals, and disturbances in serotonin systems have been linked to numerous psychiatric and neurological conditions, such as depression, anxiety, and disorders related to aggression.¹¹ *Drosophila* *Melanogaster* has emerged as a valuable model research organism for studying these processes due to its well-characterized serotonin system, which shares significant homology with humans.¹² Research utilizing *Drosophila* provides insights into how alterations in serotonin signaling can affect health and behavior. Previous research demonstrates the importance of serotonin, showing how overexpression of serotonin in specific brain regions has been associated with an increase in aggression¹³ and depletion of serotonin has been linked to impaired movement.¹⁴

Serotonin and *Drosophila* Social Behaviors

The creation of distinct fly lines (Gal4 38389 x UAS 25774 and Gal4 38389 x UAS 55315) enables both the visualization and optogenetic manipulation of serotonin neurons, facilitating a comprehensive investigation into the neurobiological mechanisms behind serotonergic control of behavior. The manipulation of serotonin neurons via the expression of halorhodopsin allows for the acute silencing of serotonergic signaling, providing an experimental tool to explore the relationship between serotonin activity and behavior. The acute depletion of serotonin will be the key in addressing the functional role of serotonin in aggression, movement, and food consumption.

¹¹ Jacobs, B. L., and Azmitia, E. C.. Structure and Function of the Brain Serotonin System. *Physiological reviews*, 1992. 72(1), 165–229. PMID: 1731370

¹² Carter OL, Burr DC, Pettigrew JD, Wallis GM, Hasler F, Vollenweider, FX. Using Psilocybin to Investigate the Relationship Between Attention, Working Memory, and the Serotonin 1A and 2A receptors. *Journal of Cognitive Neuroscience*, 2005. 17(10): p1497-508. PMID: 16269092.

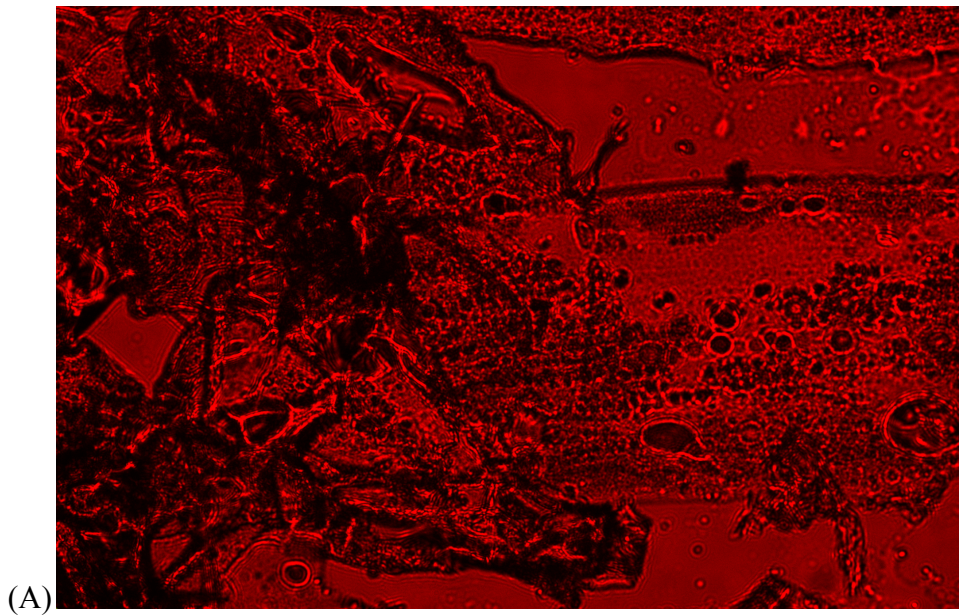
¹³ Tao, R., Rudacille, M., Zhang, G., & Ma, Z. (2014). Changes in intensity of serotonin syndrome caused by adverse interaction between monoamine oxidase inhibitors and serotonin reuptake blockers. *Neuropsychopharmacology*, 39(8), 1996–2007. PMID: 24577320.

¹⁴ Dierick HA, Greenspan RJ. Serotonin and neuropeptide F have opposite modulatory effects on fly aggression. *Nat Genet*, 2007. PMID: 17450142.

RESULTS

GAL4	UAS	Purpose
38389 - Serotonin	25774 - mCherry	Visualization
38389 - Serotonin	55315 - Halorhodopsin	Optogenetic Manipulation

Figure 1: Cell Line Generations—the GAL4 and UAS lines and their purposes. GAL4 38389 was crossed with UAS 25774 to visualize the serotonin neurons with mCherry. GAL4 38389 was crossed with UAS 55315 in order to optogenetically manipulate the flies using halorhodopsin channels.



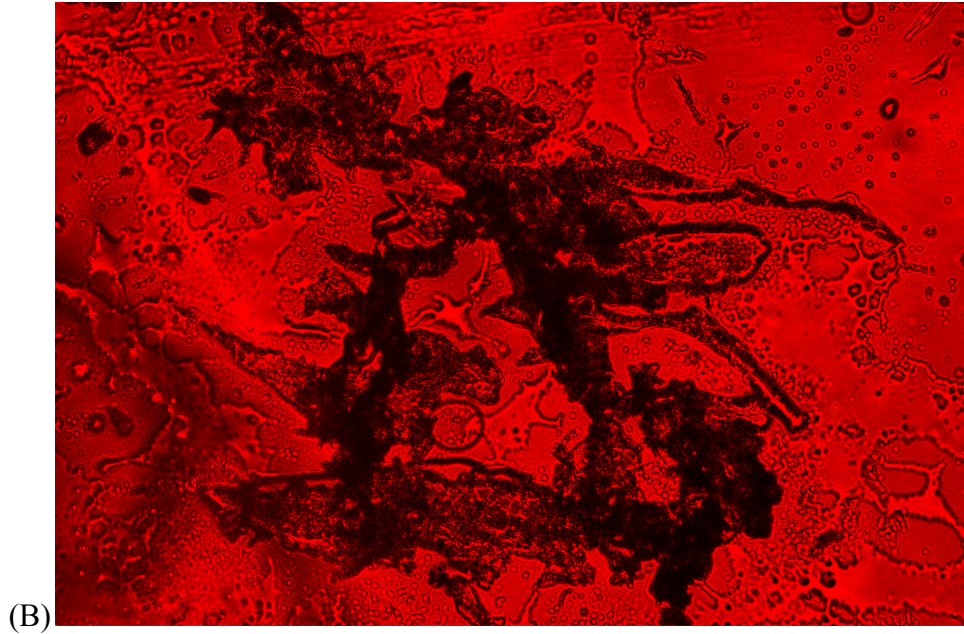


Figure 2: *Drosophila* Serotonergic System. (A) 20X magnification (B) 10X magnification. Images of the fly brain, using a red filter attachment, the cells that fluoresce red are serotonin neurons, indicating where the system of serotonergic neurons are spatially located.

Fighting Behavior

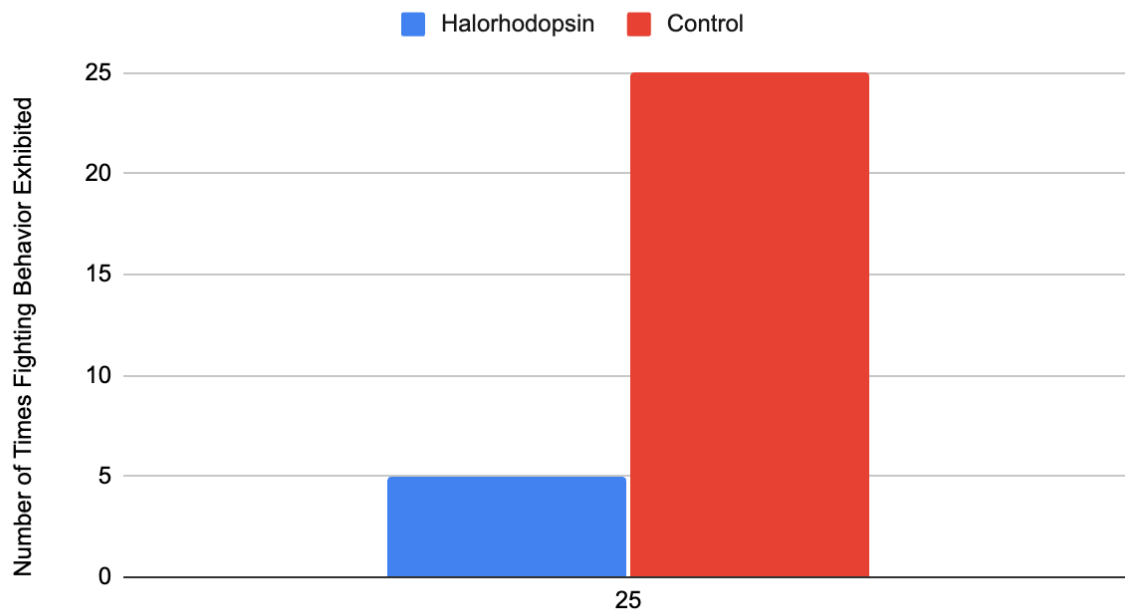


Figure 3: Fighting Behavior Comparison Between Halorhodopsin and Control *Drosophila*. In order to measure social behavior, a tally of fighting behaviors were compared

across control and halorhodopsin flies. The halorhodopsin flies have exhibited less fighting behaviors, suggesting a loss in motivation associated with depressive symptoms.

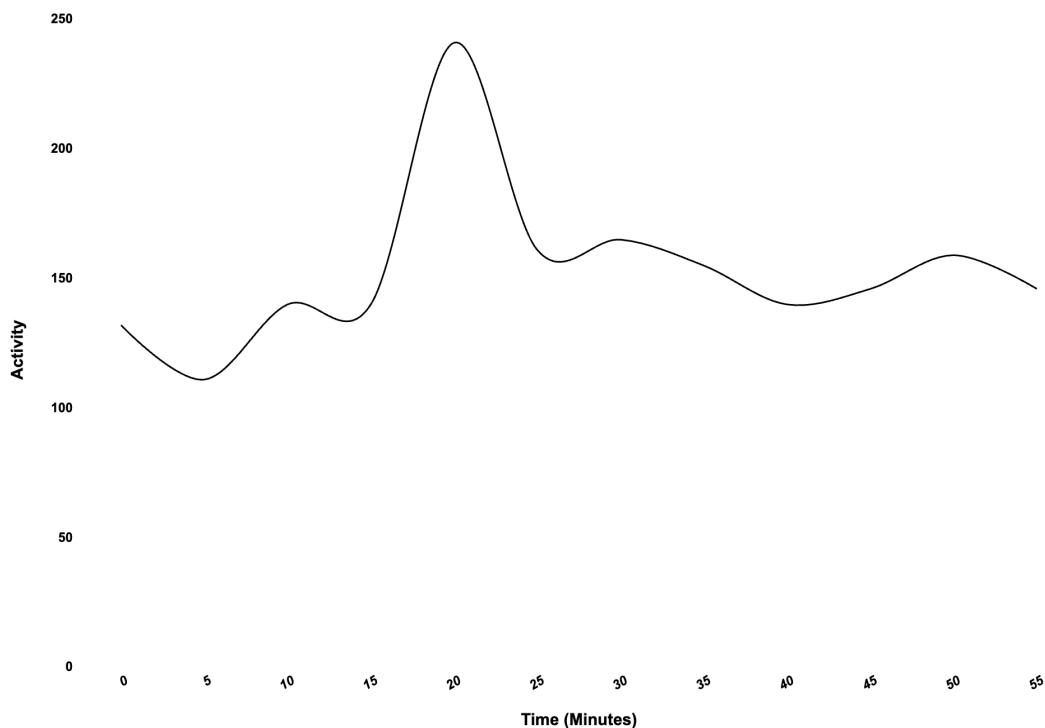


Figure 4 A: Drosophila Activity Monitor (DAM) Data. Monitoring Movement.

Control Drosophila Activity Over 55 Minutes. Template data collected from 6 vials of *Drosophila* was collected every 5 seconds and condensed into 5 minute increments over a period of 55 minutes. The graph above measures the average amount of DAM beam breaks per 5 minutes caused by fly movement across all the vials. The most dramatic peaks in activity occur at $t=10$, 20, 30, and 50 minutes with $t=20$ being the largest peak. These peaks correspond to regular locomotive behavior, where average activity of all vials over the 55 minute time period is 153 beam breaks. On average, the *Drosophila* without acute serotonin inhibition experienced more overall movement than those that had been depressed.

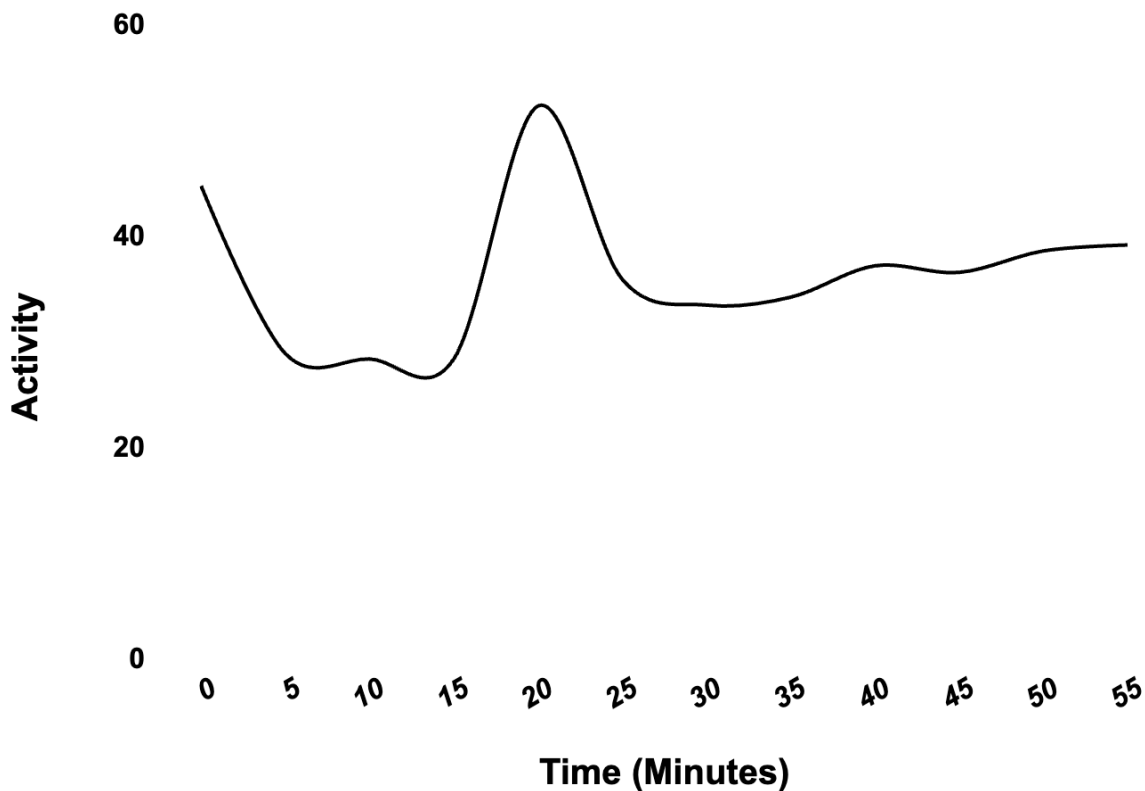


Figure 4 B: Drosophila Activity Monitor (DAM) Data. Monitoring Movement.

Average Serotonin Inhibited Drosophila Activity Over 55 Minutes. Template data collected from 6 vials of Drosophila was collected every 5 seconds and condensed into 5 minute increments over a period of 55 minutes. The graph above measures the average amount of DAM beam breaks per 5 minutes caused by fly movement across all the vials. The most dramatic peaks in activity occur at t=10, 20, 40, 50 hours with t=6 being the largest peak. These peaks correspond to regular Drosophila circadian rhythm behavior, where activity increases around these dusk and dawn adjacent time periods. On average, the Drosophila with acutely inhibited serotonin had less movement than those in the control group (in the dark), with 36.57552083 beam breaks over the 55 minute time period.

Light Absorbance (Control)	Light Absorbance (Halorhodopsin)
-2.107 AU	-1.305 AU

Figure 5: Eating Behavior. Light absorbance measurements of ingested dye in control and halorhodopsin-expressing Drosophila. Flies were given access to dyed food for 55 minutes in DAM vials. Drosophila abdomen were then isolated to measure spectrophotometry light absorbance. Control Drosophila exhibited a higher absorbance value (-2.107 AU) compared to

the halorhodopsin *Drosophila* (-1.305 AU), indicating greater food consumption by the control group. The reduced absorbance in halorhodopsin flies suggests anhedonia, as these flies consumed less food compared to controls.

EXPERIMENTAL DESIGN

Specific Aim 3: Observing Anhedonic Behavior

Experiment 1a: Examining the social behavior of mutated *Drosophila*

Rationale: Social behaviors are a defining characteristic of many neurological disorders. By examining female aggressive interactions through specifically designed fighting chambers will quantify the initiation and persistence of fighting behaviors, the establishment of dominance hierarchies, and resource defense strategies.

Design: Female *Drosophila* were isolated from the pupae stage to prevent socialization history that would affect their behavior during the experiment. The experiment was performed in a standard arena with a fresh food cup, which the female will fight over. The isolated females were anesthetized using CO₂ then gently aspirated into the fighting chamber at the same time. Behaviors were recorded immediately by scoring wing threats, holds, fencing, lunging, tussle, and tossing.

Results, Interpretations, Potential Pitfalls: We expected that the acutely depressed *Drosophila* would not exhibit as much fighting behavior as the control group due to the lack of serotonin. We recognized that these experiments take considerable time and patience and that the *Drosophila* may not interact right away, if at all. Additionally, accurately observing behavior was found difficult as one normal movement could have been falsely misinterpreted as a fighting behavior. This could also be influenced by observer bias, as we are looking to prove our hypothesis correct. Another issue to consider is problems with the chamber design. The recording of the chamber was not great quality, with glue and tape blocking a complete view of the chamber. Another variable to consider, that affected all experiments, includes slight age differences in the *Drosophila* that affect behavior.

Experiment 1b: Studying *Drosophila* Locomotion

Rationale: The *Drosophila* Activity Monitor (DAM) provided a technological platform that quantified locomotive behavior in the female *Drosophila* over a specific period of time. This was important for gathering high resolution behavioral data to analyze daily activity patterns without researcher influence or bias. By using DAM analysis, we directly linked the genetic modifications of acutely depleted serotonin to quantifiable changes in movement patterns.

Design: 2 DAM monitors were set up, one that monitored modified flies and one that monitored the control group of flies. The climbing assays were run over a period of 55 minutes. Before and after the experiment, flies were allowed a 15 minute resting period. Data was collected and interpreted to observe the amount of beam breaks that occurred during the experiment.

Results, Interpretations, Potential Pitfalls: The DAM was run after the fighting chamber experiment, so 15 minutes of rest may not have been sufficient time for the *Drosophila* to recover and express normal locomotor behaviors. The *Drosophila* may express decreased climbing speed or reduced overall movement due to overexertion rather than serotonergic manipulations. Another consideration is the time of day that this experiment is run. The natural circadian rhythm of *Drosophila* may impact the movement that occurs during the 55 minutes of data collection. Another variable to consider, that affects all experiments, includes slight age differences in the *Drosophila* that affect behavior.

Experiment 1c: Studying *Drosophila* Eating Behavior

Rationale: The cafe assay was presented to test the eating behavior of female *Drosophila* over a specific period of time. By observing the result of the eating assay, a relationship between the genetic modification and *Drosophila*'s eating behavior can be correlated.

Design: In this behavioral test, flies were fed liquid food provided in graded glass capillaries placed inside a vial. This experiment was run in the DAM capillaries. The decline of the capillary meniscus indicated the loss of food solution via evaporation and food consumption. Dark blue food dye was mixed with the prepared liquid food. The flies were starved 4 hours before experiment 1a to make sure that the fly subjects were likely to exhibit eating behavior. Access to food also drove motivation for locomotion during experiment 1b. After the 55 min DAM with complete access to dyed food, flies were anesthetized and the abdomens were isolated to observe the amount of ingested dye through spectrophotometry. This assay was also used to observe a control group for the exact same procedure for comparison.

Results, Interpretations, Potential Pitfalls: Through the cafe assay, the amount of dye observed in fly's abdomen led to the possible result of this test. The experimental *Drosophila* did exhibit a decrease in food consumption, as the absorbance recorded from the fly abdomens was lower than those of the control. If the fly shows a decrease in food intake, a possible cause could be that the starvation time is not enough for the experiment to run ideally. If a fly was not starved enough, it would not eat as much food. Another factor to affect the outcome could be the temperature. Too high or too low of the environmental temperature will also cause a significant food intake difference. Some other genetic deficits in its gene line or the time of the day to present the assay may also affect the outcome of the experiment.

METHODS/TIMELINE

ANIMAL CARE/LINE GENERATION

Drosophila Melanogaster will be raised in vials containing a molasses (B2) mixture of food—86% water, 0.574% agar, 6.30% cornmeal, 1.52% yeast, 4.65% dry molasses, 0.39% propionic acid, 0.15% methylparaben, and 0.52% ethanol. Two crosses will be created: Gal4 38389 with UAS 25774 (serotonin with mCherry visualization) and Gal4 38389 with UAS 55135 (serotonin with halorhodopsin). They will be kept in a temperature controlled incubator with a 12:12 hour light to dark cycle. The female flies will be kept in isolation chambers. Approximately four hours before experimentation, they will be food-deprived.

BEHAVIORAL ASSAY DESCRIPTION

Several behavioral assays will be used to observe the resulting outcome from these genetic crosses. These assays will be utilized to study *Drosophila* locomotion, eating habits, and fighting motivation. Each assay will last approximately 30 minutes, as the same flies will be used for each experiment and all in the same day. Before testing, the mutated flies will be isolated in a drawer of yellow light for one hour, in order to acutely deplete serotonin prior to the assays. During this time, approximately four hours before the experiment, the flies will be starved to encourage feeding behaviors during the experiment. We will also isolate the female flies so that they have no prior socialization history that would affect their behavior. The first experiment is the social aggression assay. Aggression assays are performed in standard arenas, which are wells in a plate. Prior to fighting assays, fresh food cups, which the females will fight over, will be prepared and isolated females will be anesthetized with CO₂. The females will then be aspirated into the fighting chamber simultaneously and behavior will be recorded immediately, scoring the common fighting behaviors of wing threats, holds, fencing, lunging, tussle, and tossing. After the fighting chamber, remaining females will be given a 15 minute period of rest before proceeding to the next assay.

The last assay will combine the CAFE assay with a movement assay. By placing food colored with dye at the end of the tube and placing the *drosophila* inside the DAM, we can simultaneously monitor movement and eating. Climbing assays will be used to detect the activity of flies over a period of 55 minutes. Flies will be transferred into the DAM and data will be collected that counts the amount of beam breaks over that period of time. After the climbing assay, we will keep the flies in the fridge—preserving the dye inside their stomachs and putting them to sleep. We will isolate the abdomens and use spectrophotometry to observe food consumption via the amount of light passed through the abdomen. The more food that is consumed, the less light that will pass through.

EXPERIMENTAL PROTOCOL

The experiment will only use female flies. To guarantee that the female flies have not mated before testing, the females will be housed in isolation chambers individually. The experimental

flies (UAS 55135) will be exposed to yellow light (473 nm) for one hour in order to activate halorhodopsin in their serotonin neurons. The control flies will be kept in the dark. We prepared the flies by using CO₂ and transferring them to the fighting chamber that we built previously. We separate the fighting chamber into two parts with light exposed flies and light non exposed flies. Two flies will be assigned to one of six individual chambers, and their behavior will be observed and recorded for 30 minutes. We prepared the flies by using CO₂ and transferring them to dam vials, adding food coloring to the food at one end. Then, we analyzed the DAM movement using the data collected by the dams. Afterwards, we dissected the abdomens, crushed them, and used spectrophotometry to analyze food consumption.

DATA ANALYSIS

The experiment will be using DAM testing; same sex aggressive fighting chamber assays and eating behavior testing assays. DAM data will be recorded and analyzed through DAMFileScanII4X, and will be statistically analyzed and graphed on the Excel sheet. Behavioral assays will be videotaped. Individual encounters will be clipped from full-length records and these will be scored for behavioral patterns using Microsoft Excel spreadsheets.

PROJECTED TIMETABLE FOR CONDUCTING EXPERIMENTS

Week	Protocol
1-3	Creating fly crosses
4-6	Behavioral Assays

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