Potential consequences of adverse lifestyle factors on decision-making as modeled by the *Drosophila melanogaster* egg-laying process

Sabrina Camacho

Dr. Rebecca Yang, Department of Neurobiology, Duke University School of Medicine

The thesis is submitted as part of the requirement for Graduation with Distinction in Neuroscience

Duke University

Durham, North Carolina

2023

Table of Contents

Abstract

Studies have shown that lifestyle factors including impaired gut microbiome health, advanced maternal age, and a diet high in sugar may negatively impact cognitive functioning, but their effects on decision-making have not been thoroughly examined. This study aimed to describe the effects of these three factors on decision-making as well as to determine whether the mechanism behind these effects is metabolic or sensory. This was assessed using *Drosophila melanogaster* egg-laying chamber assays in which Drosophila were given two choices of substrate on which to lay their eggs: sucrose vs. plain or sucrose vs. sucrose. It was found that neither a reduced gut microbiome nor advanced maternal age influenced decision-making. A high-sugar diet resulted in increased sucrose preference. Neither a metabolic nor a peripheral sensory mechanism explained this phenotype, for ingesting just the nutritious element of sucrose nor just peripheral sensing of the sweet element of sucrose was sufficient to increase sucrose preference. An internal sensory mechanism using Gr43A neurons partially accounted for this phenotype, for the lack of internal sensor activity prevented the unfavorable assessment of sweetness, increasing the perceived value of sucrose. It can be concluded that a diet surpassing healthy sugar levels caused adverse changes in decision-making through a combination of metabolic and sensory mechanisms. This study fills the gap in research about whether lifestyle factors affect decision-making in humans and in Drosophila. The results of this study can be a motivator for people to adopt healthier diets and monitor their sugar intake.

Introduction

Could something as seemingly innocuous as your diet and gut health be negatively affecting your cognitive functioning? What about the age of your mother when you were born? These are aspects of our lives that most typically do not associate with affecting cognition and behavior despite being quite impactful. Much progress has been made in understanding the basis by which genetic mutations and serious trauma impact the structure and function of the brain. However, less is known about the brain's sensitivity to one's habits and aspects of lifestyle. Three factors that might negatively impact the brain regarding decision-making are reduction of the gut microbiome, advanced maternal age of one's mother, and excess sugar intake via a high-sugar diet. These factors have become increasingly relevant in modern society, and this study aims to provide more insight into their effects.

Research has shown that the gut microbiome affects an organism's physiology, ecology, behavior, and evolution (Heys et al., 2018). The gut microbiome is composed of hundreds of different types of bacteria, viruses, and other small organisms primarily in the small and large intestines and plays a role in imprinting the mucosal immune system and maintaining an organism's health (Caracciolo et al., 2014). A compromised gut microbiome, such as one with a low number of bacteria, can be attained through the overconsumption of processed foods as well as the overuse of antibiotics, and has been shown to negatively affect one's health and cognitive functioning. Furthermore, the presence of commensal gut bacteria was found to be essential for normal brain development—brain plasticity may be affected by such bacteria (Caracciolo et al., 2014). Caracciolo et al. in 2014 also found correlations between reduced microbiomes and poor health among long-term institutionalized elders.

The realization that environmental effects (i.e., antibiotic overuse) on members of a generation can affect their offspring has caused epigenetic research to grow in popularity.

Because more women are working now than ever before, the average childbearing age has been steadily increasing over the course of the last few decades. “Advanced maternal age” is a term coined to describe the pregnancy of a woman over the age of 35. A study in 1992 by Milner et al. found that women who gave birth over the age of 40 had higher rates of perinatal mortality and giving birth prematurely, as well as had more children with lower birth weights and congenital abnormalities. Bianco et al. found in 1996 that women who gave birth at or over the age of 40 were more likely to develop high-risk pregnancy diseases such as gestational diabetes or preeclampsia (a precursor to eclampsia, a condition characterized by seizures). Children affected by such direct or indirect afflictions, notably very low birth weights, have increased risks of impaired cognitive functioning during their lifetime (Farajdokht et al., 2017).

In the 21st century, unhealthy food choices are seemingly ubiquitous. Restaurants have gained a reputation for serving large portions of high-sugar, high-sodium food, and grocery stores have stocked their shelves with products that share the same qualities. Due to this trend, knowing the effects of high-sugar diets is becoming increasingly important. A study by Yeomans in 2017 revealed that a Western-style, high-fat, high-sugar diet leads to impaired hippocampal functioning, disrupting behavioral responses such as proper appetite control. The hippocampus is also involved in processes such as spatial memory formation. A similar study by Leigh and Morris in 2020 found that diets such as those high in sugar, are positively associated with impaired cognitive functioning, even if such diets are undertaken for a short period of time (days to weeks). A poor diet coupled with obesity was also shown to increase systemic inflammation and lead to a compromised blood-brain barrier, ultimately resulting in decreased cognitive functioning (Leigh and Morris, 2020). Evidently, unhealthy diets have drastic effects on cognition.

These three factors have also been studied in past research using *Drosophila melanogaster* animal models. The gut microbiome has been demonstrated to be of high importance in Drosophila regarding their overall health, including their development, life span, behavior, and disease resistance (Ludington and Ja, 2020). In a study in 2018 by Heys et al., the antibiotic streptomycin (used in humans to treat conditions such as tuberculosis) was fed to Drosophila to reduce their gut microbiome. This intervention negatively affected their development, resulting in longer incubation times and reduced larval sizes. A different study used the antibiotic tetracycline (used in humans to treat conditions such as cholera and malaria) and found that behavior such as mating aggression in males was depressed, which is highly reliant on being cognizant of searching for a suitable mate (Jia et al., 2021). These developmental impairments indicate that gut microbiomes are essential for proper development, and the observed behavioral impairments may extend to decision-making.

Epigenetic factors, including advanced maternal age, not only adversely affect humans, but also Drosophila. The average Drosophila lifespan is quite short, ranging from 40 to 50 days. A study by Bloch Qazi et al. in 2017 found that senescence, the state of growing old, decreased the viability and reproductive fitness of female Drosophila, which extended to their offspring. This influence of age on viability was also seen across multiple generations when female Drosophila with old mothers and old grandmothers had decreased viability (Hercus and Hoffmann, 2000). Furthermore, when both parents were aged, the fewest number of offspring were produced, and those offspring had low fitness (Mossman et al., 2019). Decreased viability and fitness due to advanced maternal age may correlate to lower birth weights, creating the same defective cognitive functioning risks as seen in humans.

Drosophila typically subsist on diets consisting of a balanced combination of corn, yeast, sucrose, and agar, but their diets can be easily manipulated. A study on the effect of a high-sugar

and high-fat diet on Drosophila found that the response of PAM-β’2 neurons (protocerebral anterior medial dopaminergic neurons that connect to the β’2 compartment of the mushroom body—an important structure for olfactory learning and memory) to sweet stimuli was reduced and delayed, meaning satiation was inhibited (May et al., 2020). Additionally, in 2022, Kelly et al. found that a high-sugar diet degraded the hunger-driven feeding response in Drosophila. Reproductive performance (i.e., number of eggs laid) was also decreased when Drosophila were on a high-sugar diet during development, which could indicate a possible negative effect on their progeny’s cognition (Klepsatel et al., 2020).

Although some research has been done on the cognitive results of a reduced gut microbiome, advanced maternal age, and a high-sugar diet, little has been done on their effects on one specific aspect of cognition—decision-making. The process of decision-making is based on assigning assumptions of value (i.e., importance) to different choices and ultimately concluding that one is best (or not the worst). The prefrontal cortex of the frontal lobe manages decision-making through executive function, a set of cognitive skills and processes also including impulse control and emotion regulation.

Previous work in the Yang Lab has shown that the egg-laying process by Drosophila is a valid model for studying value-based decisions. When Drosophila are faced with only plain substrates or only sucrose substrates on which to lay their eggs in an egg-laying chamber assay, they readily lay eggs on both; however, when faced with a choice of either a sucrose substrate or a plain substrate on which to lay their eggs, Drosophila strongly prefer the plain substrate (Yang et al., 2008). This paradigm illustrates that Drosophila actively avoid the sucrose substrate for egg-laying unless it is their only option on which to do so. Furthermore, the phenomenon is highly robust at the level of single-animal and can be assayed in a massively parallel manner,

making it uniquely advantageous to study the potential impact of how lifestyle factors impact decision-making.

This study aimed to describe the effects of a reduction in the gut microbiome, advanced maternal age of one's mother, and a high-sugar diet on decision-making as well as to determine if the underlying mechanism behind any observed effect was metabolic or sensory through the use of *Drosophila melanogaster* egg-laying chamber assays. It was hypothesized that each one of these factors would alter the decision-making behavior of Drosophila, and that the mechanism would have a sensory basis because that would allow for a more refined method of value assessment.

Methods

An overview of the process for preparing Drosophila to be put in the egg-laying chambers is laid out in Figure 1.

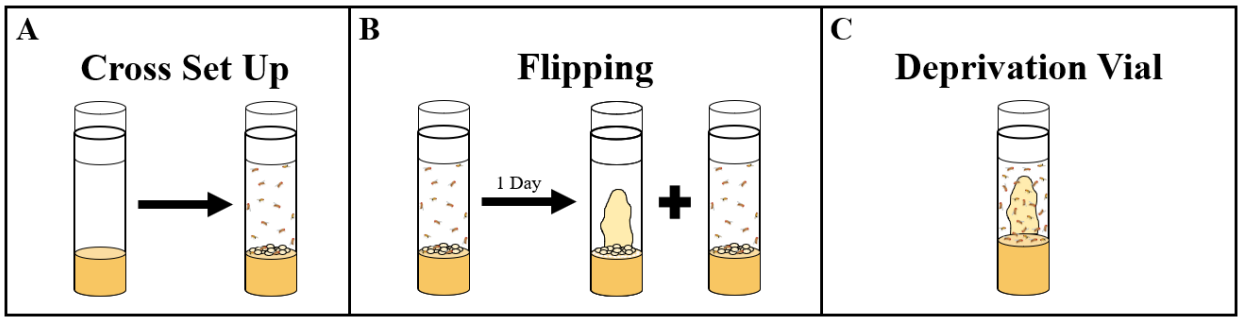

Figure 1. Preparation of Drosophila is required before using the egg-laying chamber assays. A. Crosses were set up in vials with yeast granules. B. The flipping process began one day after the cross was set up and was repeated daily for 10 days. Yeast paste was put in the old vial and yeast granules were put in the new vial. C. The deprivation vials included progenies from the previously set up crosses and treated yeast paste and food.

For the lifestyle factors experiments (reduced gut microbiome, advanced maternal age, and high-sugar diet) as well as the control for silencing Gr43A neurons, progenies from the same cross were used. To perform this cross, 10 virgin females (*w; Otd-FLP/CyO; UAS>>Kir2.1/TM6B*) and 5 males (*w; +/+; empty-split-GAL4/TM6B*) were put into a vial with yeast granules, which was repeated to have multiple crosses. The genotype of the progenies used was *w; Otd-FLP/+; UAS>>Kir2.1/empty-split-GAL4*. These progenies allowed for the normal,

ubiquitous activation of Gr43A neurons. To localize silencing of Gr43A neurons to the brain only, 10 virgin females (*w; Otd-FLP/CyO; UAS>>Kir2.1/TM6B*) were crossed with 5 males (*w; +/+; Gr43A-GAL4/TM6B*). The genotype of the progenies used was *w; Otd-FLP/+; UAS>>Kir2.1/Gr43A-GAL4*.

The UAS/GAL4 system consists of a transcriptional activator protein (GAL4) activated by a promoter that selectively binds to the upstream activation sequence (UAS) in DNA, activating the transcription of a downstream gene of interest. The altered UAS/split-GAL4 system uses two promoters before the GAL4 sequence. The UAS line contains *Kir2.1*, the downstream gene of interest that controls a potassium-gated channel that permanently inhibits neuronal activity. The driver line with split-GAL4 has a tissue-specific promoter, which is either empty or Gr43A.

The FLP/FRT system uses flippase (FLP) recombinase, which recognizes a pair of FLP recombinase target (FRT or >) sequences that flank a gene of interest. In Drosophila with the *w; Otd-FLP/+; UAS>>Kir2.1/empty-split-GAL4* genotype, the UAS/GAL4 system is inactive due to the lack of a promoter, so neither FRT nor *Kir2.1* are expressed. In Drosophila with the *w; Otd-FLP/+; UAS>>Kir2.1/Gr43A-GAL4* genotype, the UAS/GAL4 system is active due to the presence of the Gr43A promoter. When Otd-FLP, an enhancer that expresses FLP in the brain, is paired with this active system, it allows for the expression of both FRT and *Kir2.1* in Gr43A neurons in the brain only, silencing them.

Curly O (CyO) and TM6B are chromosome balancers used to produce dominant phenotypic markers in progenies that do not have the desired genotype. Progenies heterozygous for either of these balancers will have the dominant phenotype, and those homozygous for either of these balancers will not survive. Drosophila with the unwanted CyO phenotype have curly

wings, and those with the unwanted TM6B phenotype have tubby/squat bodies. These Drosophila were not used during experimentation.

Starting the day after the crosses were set up, the vials for all the crosses were flipped—the original Drosophila were put into a new vial with yeast granules, and yeast paste was put into the old vials. This was repeated for 10 days. After about 2 weeks, the progenies in the first flipped vials were adults.

For each treatment, 35 female and 10 male progenies from the vials were put into a new vial, called a deprivation vial, with a treated yeast paste and food corresponding to each separate treatment. The term "deprivation vial" is derived from the fact that once female Drosophila have access to sufficient amounts of food, they ingest it and prepare themselves to lay eggs; they compete for limited space within the vial to do so because of their reluctance to lay eggs on ground space occupied by food, larvae, or excrement. Therefore, they readily lay eggs in the chambers since they are given ample room to themselves.

For the reduced gut microbiome treatment, the antibiotic yeast paste was composed of 6 g yeast pellets, 10 mL 0.5% propanoic acid, 400 μL streptomycin, and 400 μL tetracycline. The control yeast paste was composed of 6 g yeast pellets, 10 mL 0.5% propanoic acid, and 800 μL 80% ethanol. The antibiotic food was composed of the 10 mL of original food in the vial (composed of glucose, inactivated yeast, and enriched corn meal) and 1 mL ddH_2O. The control food was composed of the 10 mL of original food in the vial and 800 μL 80% ethanol.

For the advanced maternal age treatment, crosses were set up with 30-day-old virgin females so that the old mothers would be about 1 month older than the young mothers. Normal yeast paste was used for these crosses, which was composed of 6 g yeast granules and 10 mL 0.05% propanoic acid. Normal food was also used for these crosses, which was the 10 mL of original food in the vial.

For the high-sugar diet treatment, the sugary yeast paste was composed of 6 g yeast pellets, 5 mL 2M sucrose, and 25 μL 0.5% propanoic acid. The control yeast paste was composed of 6 g yeast pellets and 10 mL 0.5% propanoic acid. The sugary food was composed of the 10 mL of original food in the vial and 1 mL 2M sucrose. The control food was composed of the 10 mL of original food in the vial and 1 mL ddH_2O.

For the sorbitol diet treatment, the sorbitol yeast paste was composed of 6 g yeast pellets, 5 mL 2M sorbitol, and 25 μL 0.5% propanoic acid. The control yeast paste was composed of 6 g yeast pellets and 10 mL 0.5% propanoic acid. The sorbitol food was composed of the 10 mL of original food in the vial and 1 mL 2M arabinose. The control food was composed of the 10 mL of original food in the vial and 1 mL ddH_2O.

For the arabinose diet treatment, the arabinose yeast paste was composed of 6 g yeast pellets, 5 mL 2M arabinose, and 25 μL 0.5% propanoic acid. The control yeast paste was composed of 6 g yeast pellets and 10 mL 0.5% propanoic acid. The arabinose food was composed of the 10 mL of original food in the vial and 1 mL 2M arabinose. The control food was composed of the 10 mL of original food in the vial and 1 mL ddH_2O.

The deprivation vials with their appropriately treated yeast paste and food were ready to be used for the egg-laying chamber assay in 4 days after the Drosophila had substantial time to mate and prepare themselves to lay eggs. To begin the egg-laying assay, chambers were set up following the protocol developed by Dr. Rebecca Yang of the Yang Lab and colleagues (Gou et al., 2016) as seen in Figure 2.

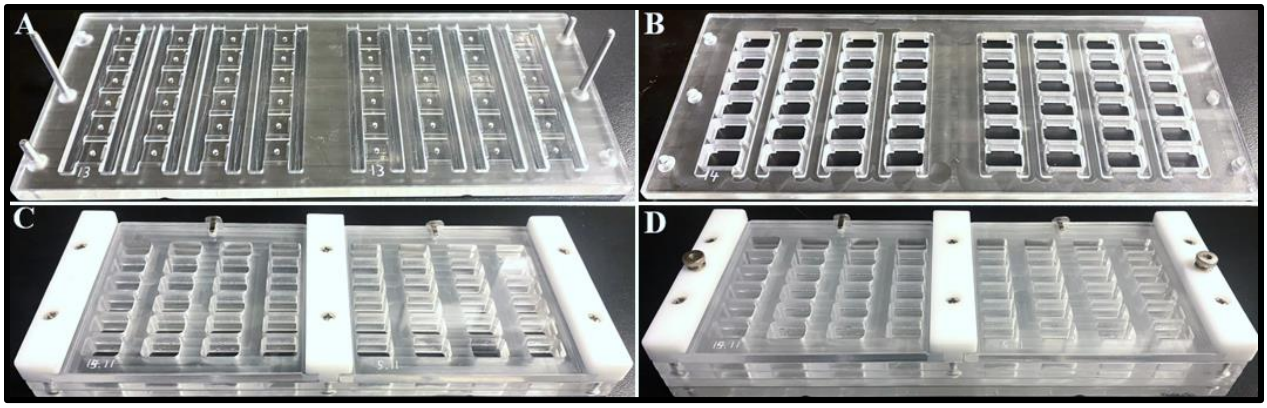

Figure 2. The egg-laying chambers are composed of three pieces. A. The bottom piece has two parallel troughs alongside each column of arenas for individual Drosophila. The left and ride troughs along each arena column differ in what substrate is contained in them (i.e., one treated with sucrose and one untreated). B. The middle piece covers up the troughs and partitions them for each arena. C. The top piece covers the arenas and the trough partitions. D. The three pieces are held together by nuts and bolts.

The troughs were filled with 965 µL of agarose with differing sucrose concentrations (one concentration on the left side and another on the right side). Sensitivity conditions (X mM sucrose vs. 0 mM sucrose) were tested to observe how Drosophila's preferences changed as sucrose concentrations increased. For these conditions, Drosophila had to detect different levels of sucrose-treated substrates when paired with plain substrates. Discrimination conditions (X mM sucrose vs. Y mM sucrose) were tested to observe to what extent differences in concentration values affected Drosophila's preferences. These conditions required Drosophila to choose between different levels of sucrose-treated substrates paired with each other. The agarose solutions were made using different amounts of 2M sucrose. For example, a 10 mL agarose solution with 50 mM sucrose was made with 250 uL 2M sucrose and 9750 uL 0.1% agarose. The plain agarose solution had nothing added to it. The agarose solution solidified to a gel-like state after a few minutes.

Control female Drosophila were put into the top 3 rows of arenas of each chamber, and treated female Drosophila were put into the bottom 3 rows of arenas of each chamber, for a total of 24 Drosophila per condition in each chamber. Once the chambers were loaded with

Drosophila, they were kept at 25ºC and 65% humidity for approximately 24 hours to allow the Drosophila to lay a substantial number of eggs without risking larval development.

After the egg-laying period, Drosophila were removed from the chambers and the egg counter program developed by Robert Alfredson of the Yang Lab was used to calculate the number of eggs laid by each Drosophila on each side of its arena as seen in Figure 3. This program works by analyzing images taken of the bottom piece of the chamber and searching for the egg shape, size, and color within each trough partition.

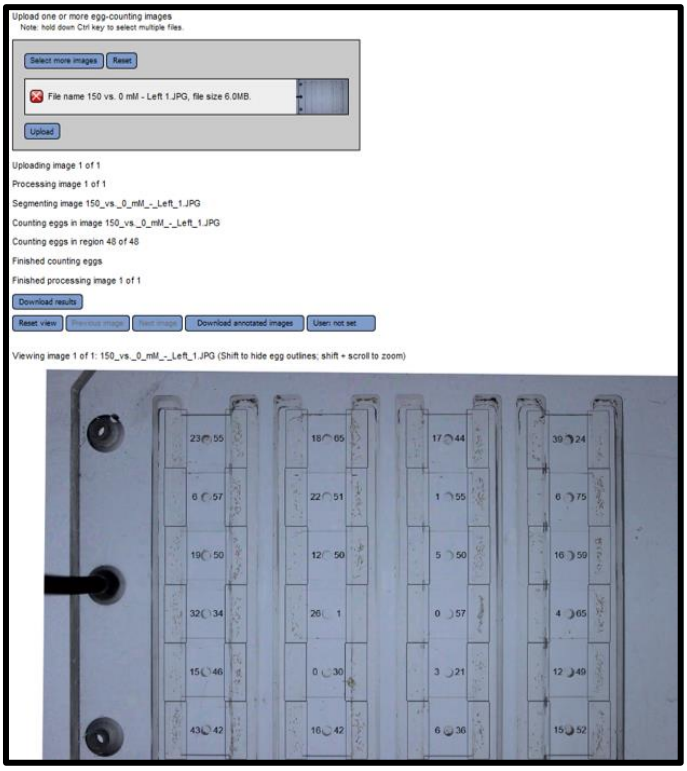

Figure 3. The egg counter program analyses chamber images to determine the number of eggs laid. This screen capture shows the results from the egg counter program for female progenies given the choice of either a 150 mM sucrose substrate (left) or a 0 mM sucrose substrate (right) on which to lay their eggs. The top left-most Drosophila laid 23 eggs on the 150 mM sucrose substrate and 55 eggs on the 0 mM substrate.

These egg values were then used in the following equation to calculate the egg-laying preference index (PI) for the higher sucrose concentration side of the arena for each Drosophila that laid at least 10 total eggs:

PI = [# of eggs on higher sucrose concentration side - # of eggs on lower sucrose concentration side] / total # of eggs

Possible PI values ranged from -1.00 to 1.00. The more positive the PI, the more Drosophila preferred the higher sucrose concentration substrate, and the more negative the PI, the more Drosophila preferred the lower sucrose concentration substrate.

Unpaired t-tests with Welch's correction (because variances were unequal) were performed between the controls and treatments for each sensitivity and discrimination condition.

Results of Adverse Lifestyle Factors

The overall trend seen for all three treatments was that for the sensitivity conditions in which Drosophila were given the choice of either a substrate with a sucrose concentration under 50 mM or a plain substrate, Drosophila had positive PIs. Once the sucrose concentration reached 50 mM, the PIs steeply dropped to the negatives. For all the discrimination conditions under each treatment, Drosophila had negative PIs.

No significant differences were found between the control and antibiotic treatment for any sensitivity or discrimination conditions tested, as seen in Figure 4.

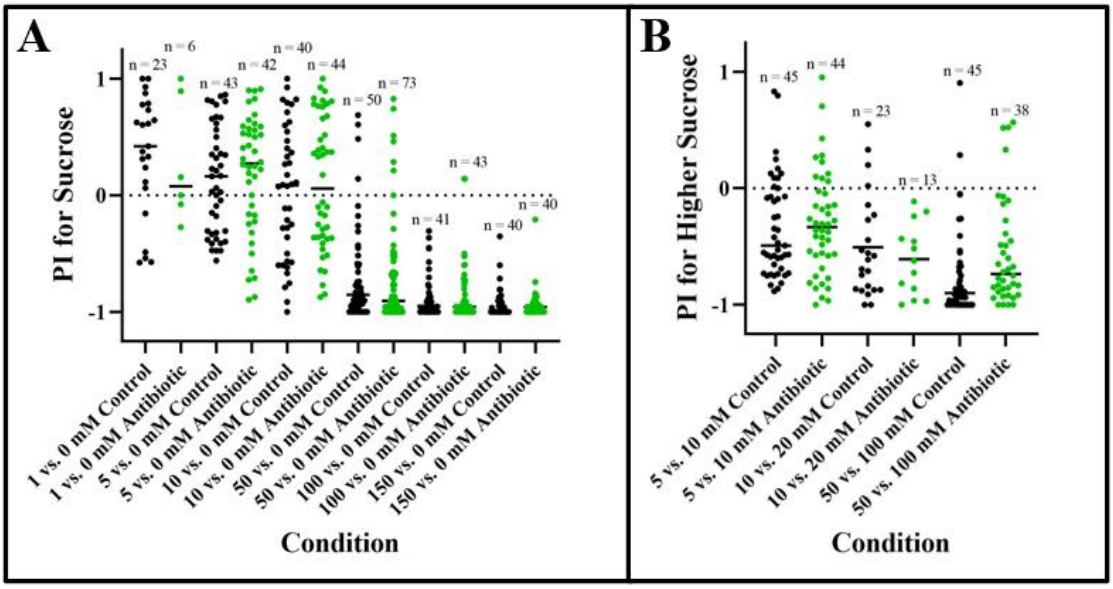

Figure 4. Reduction of the gut microbiome did not affect sucrose vs. plain decisions or sucrose vs. sucrose decisions made by Drosophila. No conditions were significantly different.

No significant differences were found between the control and advanced maternal age treatment for any sensitivity or discrimination conditions tested, as seen in Figure 5.

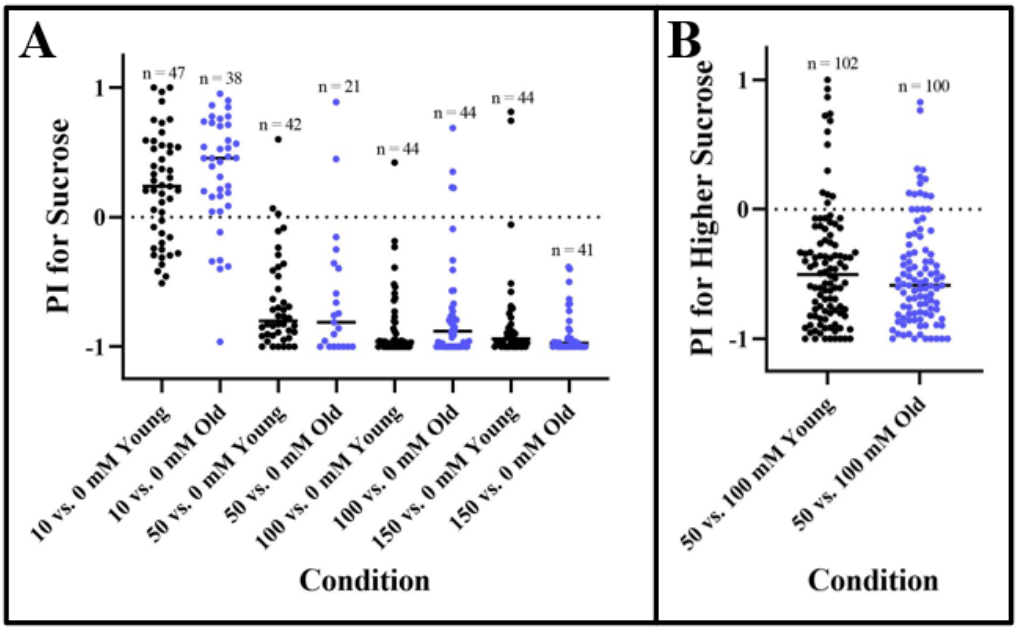

Figure 5. Advanced maternal age did not affect sucrose vs. plain decisions or sucrose vs. sucrose decisions made by Drosophila. No conditions were significantly different.

Two significant differences were found between the control and high-sugar diet treatment. They were for the sensitivity conditions of 50 vs. 0 mM sucrose ($p = 0.0047$) and 150 vs. 0 mM sucrose ($p = 0.0256$), as seen in Figure 6. For both conditions, the PI of Drosophila on the high-sugar diet increased.

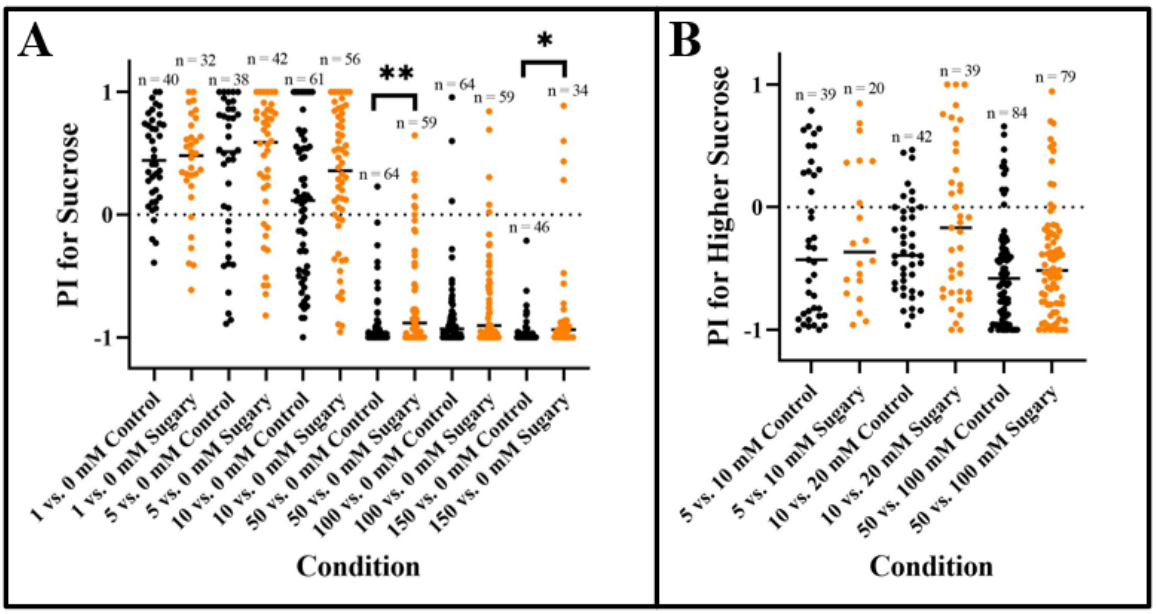

Figure 6. A high-sugar diet increased the sucrose preference of Drosophila. Two sensitivity conditions were significantly different: 50 vs. 0 mM sucrose ($p = 0.0047$) and 150 vs. 0 mM sucrose ($p = 0.0256$).

Results of Mechanism Underlying Sucrose Preference Seen Under High-Sugar Diet

The high-sugar diet treatment was the only treatment that resulted in changes in the decision-making behavior of Drosophila. This diet induced an increased preference for sucrose during the egg-laying process. Sucrose has both metabolic and sensory properties. Metabolically, it provides nutrition and thereby increases ATP levels. Sensorially, it activates both peripheral and internal sensory receptor neurons. Further experimentation was performed to determine if the mechanism underlying the increased preference for sucrose Drosophila expressed when fed a high-sugar diet was metabolic or sensory. The conditions tested were those that resulted in significant differences under a high-sugar diet (50 vs. 0 mM and 150 vs. 0 mM).

Feeding Drosophila a sorbitol-based diet (has nutritional value but is not sweet) allowed for the ability to determine if ingesting nutrition is what impacted Drosophila's value assignments when making egg-laying decisions, leading to an increased sucrose preference. No significant differences were found between the control and sorbitol diet treatment for the two conditions tested, as seen in Figure 7.

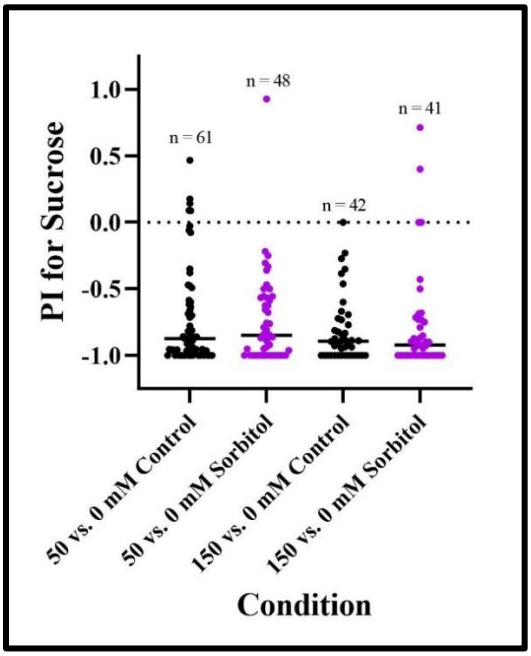

Figure 7. Metabolism does not explain the increased sucrose preference seen under a high-sugar diet. No conditions were significantly different.

Feeding Drosophila an arabinose-based diet (sweet but has no nutritional value) allowed for the ability to determine if the peripheral sensing of sweetness is what led to an increased sucrose preference. No significant differences were found between the control and arabinose diet treatment for the two conditions tested, as seen in Figure 8.

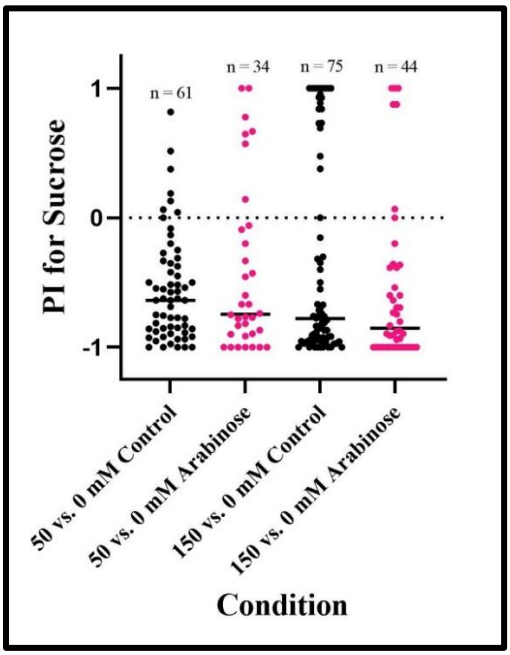

Figure 8. Peripheral sensing does not explain the increased sucrose preference seen under a high-sugar diet. No conditions were significantly different.

Gr43A is a type of gustatory receptor neuron in chemosensory organs and the brain that senses levels of fructose (a resulting subunit after the breakdown of sucrose) in blood (Musso et al., 2021). Figure 9 by Miyamoto et al. in 2012 shows a schematic of the Gr43A activation process.

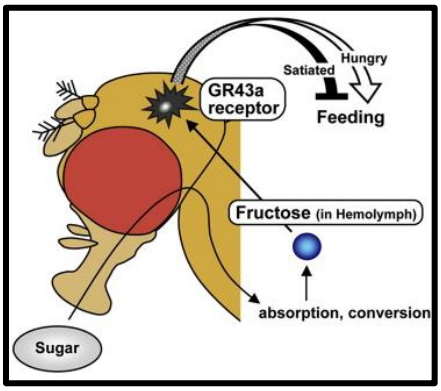

Figure 9. Gr43A receptor neurons sense fructose levels in the hemolymph of blood. Once sucrose is broken down into its fructose and glucose subunits, Gr43A gustatory receptor neurons sense fructose levels in blood.

Silencing the Gr43A neurons in the brains of Drosophila on a high-sugar diet allowed for the ability to determine if the internal sensing of fructose (i.e., sweetness) by Gr43A neurons is what led to an increased sucrose preference. Four significant differences were found: one between the empty-split-GAL4 and Gr43A-GAL4 treatment for 50 vs. 0 mM under the control diet ($p = 0.0037$), one between the empty-split-GAL4 and Gr43A-GAL4 treatment for 50 vs. 0 mM under the high-sugar diet ($p = 0.0062$), one between the empty-split-GAL4 and Gr43A-GAL4 treatment for 150 vs. 0 mM under the control diet ($p = 0.0309$), and one between the control diet and the high-sugar diet under Gr43A-GAL4 for 50 vs. 0 mM ($p = 0.0198$), as seen in Figure 10.

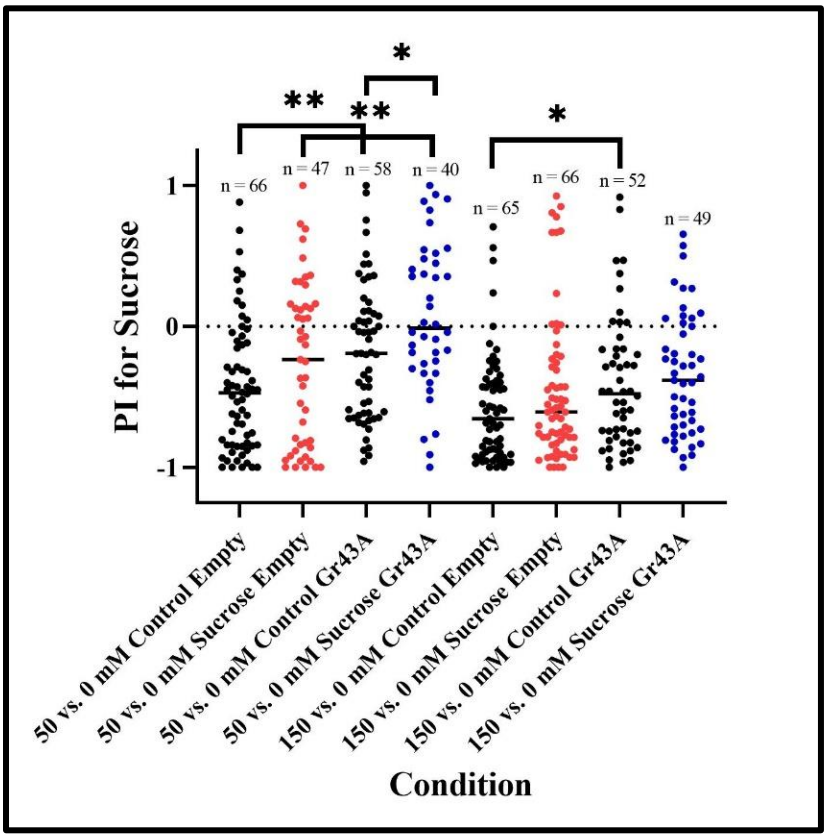

Figure 10. Internal sensing partially explains the increased sucrose preference seen under a high-sugar diet. Four conditions were significantly different: between empty-split-GAL4 and Gr43A-GAL4 for 50 vs. 0 mM under the control diet ($p = 0.0037$), between empty-split-GAL4 and Gr43A-GAL4 for 50 vs. 0 mM under the high-sugar diet ($p = 0.0062$), between empty-split-GAL4 and Gr43A-GAL4 for 150 vs. 0 mM under the control diet ($p = 0.0309$), and between the control diet and the high-sugar diet under Gr43A-GAL4 for 50 vs. 0 mM ($p = 0.0198$).

Discussion

Neither a reduced gut microbiome nor the advanced maternal age of progenies' mothers altered Drosophila's decision-making behavior, but a high-sugar diet increased Drosophila's sucrose preference when deciding where to lay their eggs. More specifically, this change was only seen in the 50 vs. 0 mM sucrose condition and the 150 vs. 0 mM sucrose condition and was quite minimally higher than the control despite being significant. The difference of 100 mM of sucrose between these conditions suggests that Drosophila may have very specified preferences, which cannot be generalized to being distinctly low, moderate, or high.

It can be concluded that metabolism alone does not account for the increase in sucrose preference seen in Drosophila fed a high-sugar diet. Ingesting just nutrition did not yield the same change (the sorbitol-based diet had no effect on PI), implying that the nutritious element of sucrose is not sufficient alone to induce an increase in sucrose value and preference. This may be temporally dependent, for it is unknown whether the preference of sucrose would change if Drosophila were fed a sorbitol-based diet for their entire lifespan, rather than just before the decision-making process.

Furthermore, it can be concluded that peripheral sensing of sweetness alone does not account for the increased sucrose preference seen. Sensing sweetness in peripheral sensory neurons did not yield the same change (the arabinose-based diet had no effect on PI), implying that the peripheral sensing of the sweet element of sucrose is not sufficient alone to induce an increase in sucrose value and preference. This may also be temporally dependent for the same reasons.

Lastly, it can be concluded that internal sensing of fructose (i.e., sweetness) in the bloodstream via Gr43A neurons alone can partially account for the increased sucrose preference seen. The lack of internal sensor activity may have prevented proper assessment of value by

preventing the unfavorable assessment of sweetness, thereby increasing the perceived value of sucrose. This is indicated by the significant increase in sucrose preference between the control diet and the high-sugar diet when Gr43A neurons were silenced for the 50 vs. 0 mM condition. The preference for sucrose also increased under both just the control diet and just the high-sugar diet for multiple conditions. This increase seen under the control diet was unexpected because there was no sucrose for Drosophila to sense, supporting the notion that Drosophila's value assessment ability may have been altered in a way that affects the assessment of more than just sweetness. Therefore, it can be concluded that a diet surpassing healthy sugar levels will cause adverse changes in cognitive functioning to where one may prefer qualities and aspects they would not normally through a combination of both metabolic and sensory mechanisms, since neither of these alone entirely explained the phenomenon seen.

The knowledge gained from these experiments fills the gap in research about whether lifestyle factors including a reduced gut microbiome, advanced maternal age of one's mother, and a high-sugar diet affect decision-making and the basis by which they do so. These results support claims that high-sugar diets negatively affect cognitive functioning and suggest that specifically, decision-making behavior is impaired. The results of this study can be a motivator for people to adopt healthier diets and monitor their sugar intake.

Limitations in this study include only feeding Drosophila one concentration of sugar and antibiotics within the respective treatments. A diet higher or lower in sugar may have yielded different PIs. Despite the types and concentrations of the antibiotics used coming from past literature, testing was not done to ensure that the gut microbiomes of Drosophila were entirely depleted, which may have skewed PIs. The diet treatments were only given to Drosophila while they were in the deprivation vials, so chronic exposure to these treatments may have resulted in different PIs. The substrates used in the diet treatments were also added to normal Drosophila

food rather than being given to Drosophila alone because Drosophila would not be able to subsist on just those substrates, so the presence of the original food may have had confounding effects on PIs. Different results may have also been obtained by using the progeny from female Drosophila of various advanced ages. Furthermore, when the Gr43A neurons were silenced, they were inactive for the entirety of Drosophila's lifespans rather than just during the egg-laying assays, so this inability to have ever internally sensed fructose may have influenced their decision-making process in a way that is not reflective of the actual phenomenon.

Further research can be done to expand the range of diet compositions fed to Drosophila (such as including high levels of fat) as well as the concentration of the added substrate, to see if other diets affect PIs. Varying antibiotic types and concentrations can be used to investigate whether they affect gut microbiomes, and thereby decision-making, differently. Diet treatments can be given to Drosophila over the course of their entire lives to see if any changes in PI seen are temporally dependent. Substrates that were added to the normal food can be given to Drosophila alone to determine if the absence of normal food affects PI. Female Drosophila of varying advanced ages can be utilized to determine if there are in fact maternal age-dependent effects on PI, but at ages younger or older than the one tested in this study. For further exploration into the mechanistic basis behind the changes in PI seen under a high-sugar diet, Gr43A neurons can be temporally silenced only when Drosophila are undergoing the egg-laying decision process in the chamber assays to observe whether the timing of the silencing affects PI. Sex differences could also be studied if the egg-laying chamber assay were changed to a task that both males and females partake in.

References

Bianco, A., Stone, J., Lynch, L., Lapinski, R., Berkowitz, G., & Berkowitz, R. L. (1996). Pregnancy outcome at age 40 and older. *Obstetrics and gynecology*, *87*(6), 917–922. https://doi.org/10.1016/0029-7844(96)00045-2

Bloch Qazi, M. C., Miller, P. B., Poeschel, P. M., Phan, M. H., Thayer, J. L., & Medrano, C. L. (2017). Transgenerational effects of maternal and grandmaternal age on offspring viability and performance in Drosophila melanogaster. *Journal of Insect Physiology*, *100*, 43–52. https://doi.org/10.1016/j.jinsphys.2017.05.007

Caracciolo, B., Xu, W., Collins, S., & Fratiglioni, L. (2014). Cognitive decline, dietary factors and gut–brain interactions. *Mechanisms of Ageing and Development*, *136–137*, 59–69. https://doi.org/10.1016/j.mad.2013.11.011

Farajdokht, F., Sadigh-Eteghad, S., Dehghani, R., Mohaddes, G., Abedi, L., Bughchechi, R., Majdi, A., & Mahmoudi, J. (2017). Very low birth weight is associated with brain structure abnormalities and cognitive function impairments: A systematic review. *Brain and cognition*, *118*, 80–89. https://doi.org/10.1016/j.bandc.2017.07.006

Gou, B., Zhu, E., He, R., Stern, U., & Yang, C. H. (2016). High Throughput Assay to Examine Egg-Laying Preferences of Individual *Drosophila melanogaster*. *Journal of Visualized Experiments*, *109*. https://doi.org/10.3791/53716

Hercus, M. J., & Hoffmann, A. A. (2000). Maternal and grandmaternal age influence offspring fitness in Drosophila. *Proceedings. Biological sciences*, *267*(1457), 2105–2110. https://doi.org/10.1098/rspb.2000.1256

Heys, C., Lizé, A., Blow, F., White, L., Darby, A., & Lewis, Z. J. (2018). The effect of gut microbiota elimination in *Drosophila melanogaster*: A how-to guide for host-microbiota studies. *Ecology and Evolution*, *8*(8), 4150–4161. https://doi.org/10.1002/ece3.3991

Jia, Y., Jin, S., Hu, K., Geng, L., Han, C., Kang, R., Pang, Y., Ling, E., Tan, E. K., Pan, Y., & Liu, W. (2021). Gut microbiome modulates Drosophila aggression through octopamine signaling. *Nature Communications*, *12*(1). https://doi.org/10.1038/s41467-021-23041-y

Kelly, K. P., Alassaf, M., Sullivan, C. E., Brent, A. E., Goldberg, Z. H., Poling, M. E., Dubrulle, J., & Rajan, A. (2022). Fat body phospholipid state dictates hunger-driven feeding behavior. *ELife*, *11*. https://doi.org/10.7554/elife.80282

Klepsatel, P., Knoblochová, D., Girish, T. N., Dircksen, H., & Gáliková, M. (2020). The influence of developmental diet on reproduction and metabolism in Drosophila. *BMC Evolutionary Biology*, *20*(1), 93. https://doi.org/10.1186/s12862-020-01663-y

Leigh, S. J., & Morris, M. J. (2020). Diet, inflammation and the gut microbiome: Mechanisms for obesity-associated cognitive impairment. *Biochimica Et Biophysica Acta (BBA) – Molecular Basis of Disease*, *1866*(6), 165767. https://doi.org/10.1016/j.bbadis.2020.165767

Ludington, W. B., & Ja, W. W. (2020). Drosophila as a model for the gut microbiome. *PLoS pathogens*, *16*(4), e1008398. https://doi.org/10.1371/journal.ppat.1008398

May, C. E., Rosander, J., Gottfried, J., Dennis, E., & Dus, M. (2020). Dietary sugar inhibits satiation by decreasing the central processing of sweet taste. *ELife*, *9*. https://doi.org/10.7554/elife.54530

Milner, M., Barry-Kinsella, C., Unwin, A., & Harrison, R. (1992). The impact of maternal age on pregnancy and its outcome. *International Journal of Gynecology &Amp; Obstetrics*, *38*(4), 281–286. https://doi.org/10.1016/0020-7292(92)91019-k

Miyamoto, T., Slone, J., Song, X., & Amrein, H. (2012). A fructose receptor functions as a nutrient sensor in the Drosophila brain. *Cell*, *151*(5), 1113–1125. https://doi.org/10.1016/j.cell.2012.10.024

Mossman, J. A., Mabeza, R. M. S., Blake, E., Mehta, N., & Rand, D. M. (2019). Age of Both Parents Influences Reproduction and Egg Dumping Behavior in Drosophila melanogaster. *Journal of Heredity*, *110*(3), 300–309. https://doi.org/10.1093/jhered/esz009

Musso, Y., Junca, P., & Gordon, M. D. (2021). A neural circuit linking two sugar sensors regulates satiety-dependent fructose drive in Drosophila. *Science Advances*, *7*(49). https://doi.org/abj0186

Yang, C. H., Belawat, P., Hafen, E., Jan, L. Y., & Jan, Y. N. (2008). *Drosophila* Egg-Laying Site Selection as a System to Study Simple Decision-Making Processes. *Science*, *319*(5870), 1679–1683. https://doi.org/10.1126/science.1151842

Yeomans, M. (2017). Adverse effects of consuming high fat–sugar diets on cognition: Implications for understanding obesity. *Proceedings of the Nutrition Society, 76*(4), 455-465. doi:10.1017/S0029665117000805