Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Alexander Koeppel April 3, 2025

Behavioral and Neural Mechanisms of Decision-Making in *Drosophila*

by

Alexander Koeppel

Anita V. Devineni
Adviser

Neuroscience and Behavioral Biology

Anita V. Devineni, PhD

Adviser

Patrick Cafferty, PhD

Committee Member

Alex Grizzell, PhD

Committee Member

Behavioral and Neural Mechanisms of Decision-Making in *Drosophila*

By

Alexander Koeppel

Anita V. Devineni
Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2025

Abstract

Behavioral and Neural Mechanisms of Decision-Making in *Drosophila*

By Alexander Koeppel

Decision-making is a crucial cognitive process that allows humans and animals to enact behavioral responses appropriate to environmental stimuli. This thesis investigates how *Drosophila melanogaster* make value-based decisions during foraging by examining stimulus memorization, stimulus comparison, and the underlying neural mechanisms of decision-making. Food-choice behavioral assays partially support a reinforcement learning-based model of memory-informed decision-making and show that flies compare and integrate stimuli using divisive rather than subtractive differences. Optogenetic silencing of PAM dopaminergic neurons yielded inconclusive results, likely due to experimental limitations. By offering support for reinforcement learning-based and divisive models of foraging, as a whole, our findings shed light on the complex decision-making behaviors of *Drosophila*, and lay the groundwork for future studies on the neural circuitry of decision-making.

Behavioral and Neural Mechanisms of Decision-Making in *Drosophila*

By

Alexander Koeppel

Anita V. Devineni
Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2025

Acknowledgements

I want to thank my advisor, Anita Devineni, and my mentor, Marco Peña Garcia for their perpetual support, advice, and guidance. This project would not have been possible without their help. I am also grateful for lab members Trinity Pruitt, Lam Nguyen, Sydney Walker, Yuzhen Chen, Elle McCall, and Anna Perry for their general support around the lab, especially flipping flies in my absence.

I also want to thank my committee members, Anita Devineni, Patrick Cafferty, and Alex Grizzell. This thesis marks an important milestone in my academic path, and I am fortunate to have them providing feedback and insights on my work, in order to create the best possible final work.

Table of Contents

Introduction

1.1 Background

The ability to make informed decisions is crucial for the survival and efficient function of both humans and non-human animals. In humans, abnormal decision-making is a core feature of various neuropsychiatric disorders.[1] This includes conditions of increasing medical interest, such as Substance Abuse Disorders, which reflect impaired decision-making that stems from an inability to accurately compare the reward versus harm caused by inappropriate drug self-administration.[1][2] Abnormal decision-making is also prevalent in other conditions, such as Schizophrenia and Alzheimer's disease, where decision-making involving trade-offs between effort and reward is significantly impaired, which may explain diminished goal-directed behavior, a typical presentation of these conditions.[3] [4] In the same vein, impaired decision-making may limit the survival of many other organisms, as decision-making is a key feature of foraging — the process of locating, detecting, and comparing various food sources. To optimize this process, it is essential for any individual to combine memory and sensory cues into a decision-making process that identifies nutrient-rich substances while avoiding nutrient-poor or otherwise harmful substances. This link between sensory integration, memory, and decision-making ability is well documented in animal models. In mammals, recent literature highlights the medial prefrontal cortex as a specific region that facilitates the incorporation of memory and physical stimuli into decision-making.[5]

1.2 Drosophila and Drosophila Foraging as a Model System

Despite broad-stroke progress in our understanding of the general mechanistic basis of decision-making, the cellular-level neural circuitry that underlies the integration of memory and sensory information is still unknown. *Drosophila melanogaster*, the common fruit fly, provides an ideal model to study these neural circuits, especially since the comprehensive mapping of the *Drosophila* brain, known as the *Drosophila* connectome, was recently achieved.[6][7] The approximately 140,000 neurons detailed in the connectome will allow for the precise tracing and study of neurons and synaptic connections that underlie sensory-memory integration, particularly within the mushroom body — *Drosophila's* primary center for memory and sensory integration.[8] Furthermore, *Drosophila* also exhibit foraging behavior similar to many other advanced organisms.

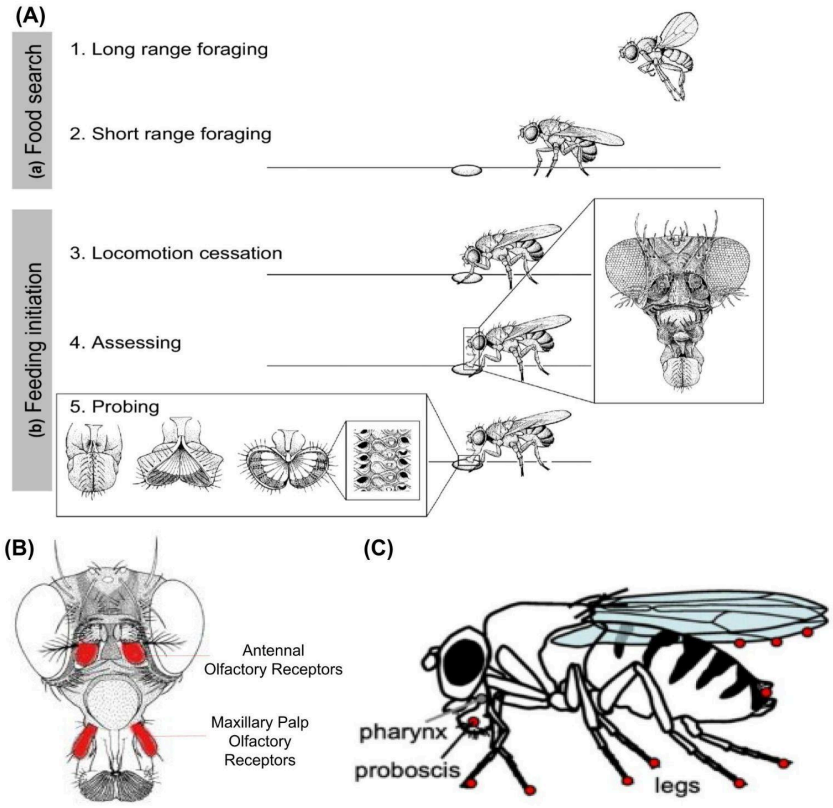

Figure 1. Stages of *Drosophila* Foraging and Associated Chemosensory Receptors

(a) Components of food search and feeding initiation — the two initial stages of *Drosophila* foraging behavior.
(b) Olfactory chemosensory receptors in *Drosophila* involved in long-range foraging behavior.
(c) Gustatory chemosensory receptors in *Drosophila* involved in short-range foraging behavior, as well as feeding initiation.

Adapted from Mahishi & Huetteroth (2019)[9], Jefferis & Luo (2005)[10], and Montell (2010).[11]

Accurately modeling foraging behavior in *Drosophila* is the first step to elucidating the neural circuitry underlying foraging decision-making in these flies. At its core, foraging in *Drosophila*, like other organisms, is a series of decisions dependent on the integration of sensory stimuli, memory, and internal state.[12] *Drosophila* contain a diverse array of chemoreceptors that can detect odors and tastants, which play different roles at various points within the foraging process. Long-range “food search” behaviors are initially driven by olfactory receptors located on the antenna and maxillary palps. However, during short-range foraging and feeding initiation, gustatory receptors take over as the primary source of chemosensation.[9] These receptors, or Gustatory Receptor Neurons (GRNs), respond to the five basic tastes present across the taste

systems of many organisms: sweet, sour, salty, bitter, and umami.[13] Individual taste signals modulate feeding behavior differently, but sugar-sensing (sweet) GRNs generally create dopaminergic appetitive signals that reinforce feeding behavior.[14] While it is important for GRNs to identify sucrose-containing food patches for foraging via these signals, in this project, we examine how *Drosophila* remember sugar signals from food patches and integrate them for comparison to one another. This allows flies to modulate their decision-making behaviors to support efficient foraging.

1.3 Memory of Gustatory Stimuli in Drosophila

As *Drosophila* explore an environment with various attractive food patches, their ability to modulate behavior solely based on encountered gustatory stimuli allows them to choose between food sources and ensure optimal nutrient uptake. This also requires memory — the ability to remember temporally distinct gustatory stimuli. Previous literature suggests that memory is present and *does* influence behavior in *Drosophila* foraging. Using optogenetic stimulation of sugar-sensing neurons, Seidenbacher et al. demonstrated that flies consistently returned to sites of sugar-neuron stimulation, indicating memory for the sites based exclusively on gustatory information.[15] Furthermore, in a study that demonstrated a similar "looping search" behavior, where flies repeatedly return to the same food source, it was demonstrated that this behavior depends on internal cues (i.e. memory), rather than newly encountered external stimuli.[16] These studies suggest that *Drosophila* may use memory of past gustatory stimuli to inform food-search foraging behavior. Previous work from our lab also demonstrates the role of memory in feeding initiation — flies briefly exposed to sugar were more likely to exhibit feeding behavior when presented with a less attractive stimulus, such as water, for a short period afterward.[17]

1.4 Models of Memory in Drosophila

The precise mechanisms by which chemosensory stimuli are encoded to memory are not known, and different models can influence decision-making behavior differently. For example, in this project, we consider the interactions of a fly moving in an environment with sucrose food patches of various discrete concentrations. Logically, it is possible that *Drosophila* remember each past gustatory stimulus independently. In other words, they may memorize as many past stimuli as different concentrations of sucrose that they have encountered. This is the core

principle of an **input retention** model of memory. Although these models do not predict precisely how these stimuli inform behavior, in broad terms, flies will feed in proportion to how a new gustatory stimulus compares to memorized previous stimuli. One possibility for the connection between a range retention model and behavior is that a fly individually compares a current stimulus to memorized previous stimuli. Then, the fly may feed if this new stimulus is "sweeter" than most previous stimuli, but continue exploring its environment if it is not.

A related model is a **range retention** model, where a fly memorizes only the highest and lowest sucrose concentrations it has encountered. This confers one specific advantage over an input retention model — it is less computationally intensive. Rather than memorizing a value for each encountered sucrose concentration, it only retains two values. In terms of this model's behavioral implications, a fly may feed if a new stimulus is at least as "sweet" as the highest previous concentration encountered, or continue exploring if the stimulus is less sweet than the lowest previous concentration. If the value lies between the two extremes, a less predictable behavioral response may occur as other factors, such as nutritional needs, may come into play.

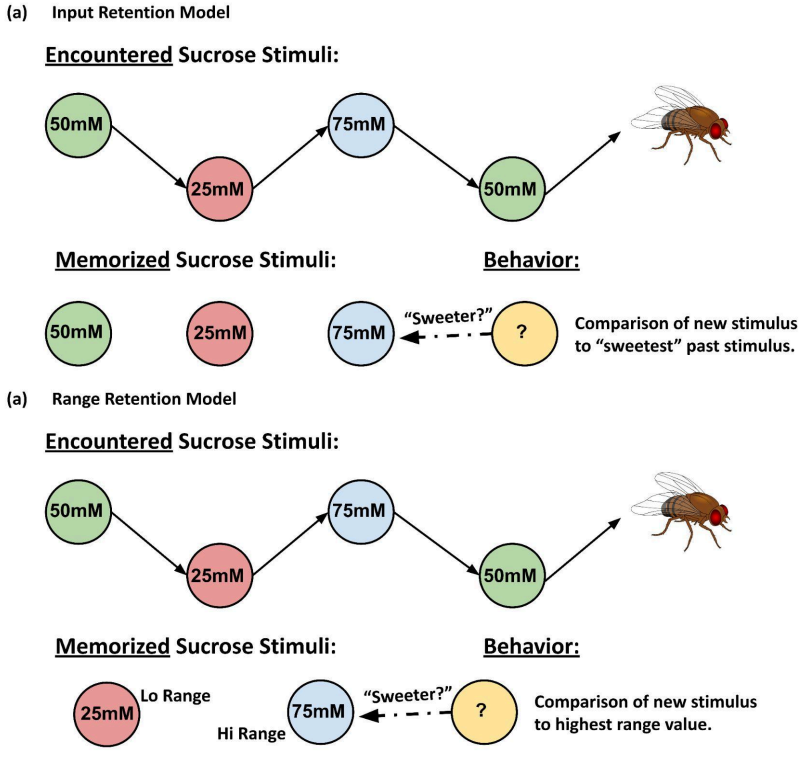

Figure 2. Input retention and range retention models of memory in *Drosophila*.
(a) Schematic of **input retention model**.
(b) Schematic of **range retention model**.

Overall, both of these models can be described as “limited context” models, as while behavior may be affected by the relative concentrations of sucrose patches according to these models, behavior would not be affected by how often the fly encounters each patch. This inability to consider the frequency of encountering different food types may be problematic, as the relative prevalence of various food sources should be an important consideration in foraging behavior. Indeed, literature concerning *Drosophila* and non-*Drosophila* models of foraging suggests more complex behavioral models, that can consider patch prevalence, may be at play.[18][19][20]

Reinforcement Learning is a model of decision-making that enables animals to update their behavior based on outcome feedback. In the context *of Drosophila* foraging, a **Reinforcement Learning-based** (RL-based) model of memory may manifest as storing a “reward prediction” for a newly encountered sucrose patch, based on the average sugar concentration of all previous sucrose encounters. Within this sucrose foraging paradigm, we may consider the patches’ relative sucrose concentrations to be equivalent to “reward.”. To inform behavior, *Drosophila* compare the discrepancy between the predicted reward for the newly encountered stimulus and its actual reward. This is termed a reward prediction error (RPE). If the actual reward is greater than the expected reward, a fly may opt to forage on the new patch, and vice versa. After the encounter, this new stimulus is also integrated into a fly’s “reward prediction” for subsequent stimuli. Complex RL-based models may also contain additional stipulations for encounter memorization — such as more recent encounters being weighted more heavily to account for the process of “forgetting.”

RL-based models of decision-making have been well-studied in the behavior of other advanced organisms. For example, pertinent research finds an RL-based model, including RPE principles, to be consistent with decision-making observed within a sucrose preference test given to mice.[18] Within *Drosophila*, a theoretical study has proposed that certain dopaminergic neurons in the fly brain likely encode RPEs within the RL-based model.[21] Unlike the aforementioned “limited context” models, in an RL-based model, the relative prevalence of different sucrose patches *does* impact memory and behavior in an RL-based model due to the mechanism by which reward prediction is calculated using past sucrose encounters.

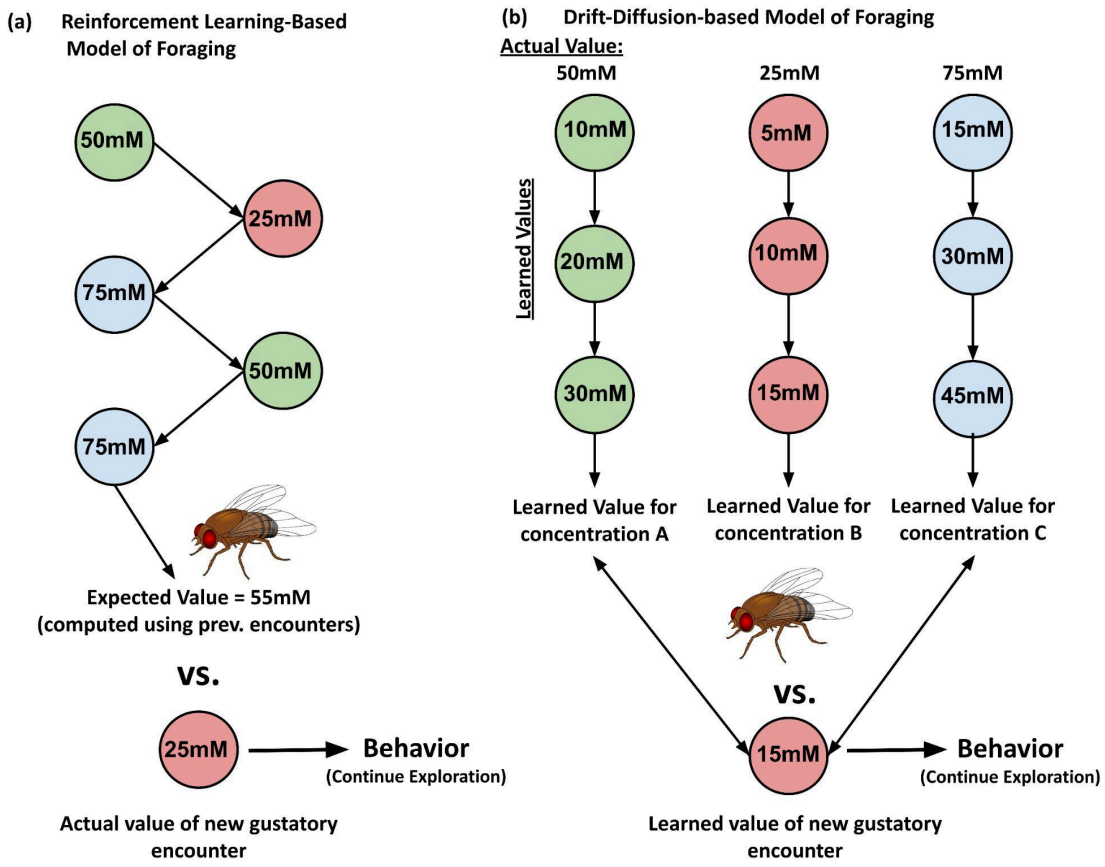

Figure 3. Reinforcement Learning and Drift Diffusion-based models of foraging.

(a) The **Reinforcement Learning-based Model** of foraging proposes that an expected reward, equivalent to sucrose concentration, is computed based on the values of previous encounters. This is then compared to the actual reward of a new gustatory encounter to form a reward prediction error, which then informs decision-making behavior. Depending on this RPE, this behavior can be either to feed, or continue exploration.

(b) The **Drift Diffusion-based Model** of foraging proposes an evidence accumulation framework, whereby the value of one class of gustatory stimulus is learned more accurately with successive encounters. The learned value of a new gustatory encounter (based on its class) is compared to the learned value of other classes of gustatory stimuli to inform behavior. If the value of comparison surpasses a pre-determined threshold, this behavior may be feeding, otherwise the fly may continue exploration.

Note: Learned values in Figure 3b are used only as an example. They are not necessarily accurate to the rate of learning in *Drosophila*.

The **Drift-Diffusion Model (DDM)** of foraging-based decision-making is an alternate model that can also consider the relative prevalence of patches. This model is related to the concept of "evidence accumulation" present in traditional DDM models — decisions are based on a given amount of evidence that has been obtained. In an environment with various sucrose patches, as previously described, *Drosophila* exhibiting a DDM-based model of behavior will

compute a “learned value” for categories of patches with distinct sucrose concentrations, which becomes more accurate to the actual value as encounters of the given concentration accumulate. For example, the more a fly interacts with a sucrose patch of a given sweetness *x*, the more this “learned value” increases, and the more it is accurate to the actual value of *x*. These “learned values” are then compared to gustatory stimuli from new patch encounters, and if this comparison surpasses a pre-determined threshold, a behavior — the decision to feed — can occur. Although the Drift-Diffusion Model was not developed specifically to explain foraging behaviors, simulation studies have applied DDM-based models to foraging.[19] [20]

A DDM-based memory model may outperform an RL-based model in specific cases—such as when a fly mostly encounters low-concentration sucrose patches and rarely finds high-concentration ones. In this scenario, an RL model may cause the fly to overlook low-concentration patches in search of an unlikely high-reward, reducing nutrient intake. A DDM model avoids this by relying on learned values rather than a single reward expectation. However, this advantage only arises under specific conditions, and a DDM-based model is always computationally intensive. Like an input retention model, a DDM-based model stores one value per category of encountered sucrose concentrations. Thus, compared to an RL-based model, which only stores one “reward expectation” value, it is relatively intensive.

1.5 Comparative Integration of Gustatory Stimuli in Drosophila

In addition to remembering previously encountered gustatory stimuli, a second component of ensuring efficient foraging is comparing chemosensory stimuli that are encountered. Our understanding of how the brain integrates chemosensory stimuli to compute absolute valence (i.e. “good” or “bad”) is relatively robust.[22][23] However, literature suggests that decisions are formed based on relative values — comparing relatively how “good” or “bad” a stimulus is relative to other stimuli.[24] It is not entirely clear how these relative values are computed or integrated to inform behavior. If we consider the previously discussed context — a fly in an environment with sucrose food patches of various concentrations — two main possibilities exist for the computation of relative value. A **subtractive model** would compare the subtractive difference between concentrations of sucrose patches. That is, forming a relative value based on the idea that “there is a 100 mM difference in sucrose concentration between patch *x* and patch *y*.” Alternatively, a **divisive model** would compare the divisive difference

between sucrose concentrations — a relative value based on the idea that "patch *x* is two times as sweet as patch *y*."

A divisive model may offer *Drosophila* more flexibility in dynamic environments with many food patches. Given that this model inherently emphasizes sensitivity to proportional differences, even small subtractive differences at lower concentrations become behaviorally significant using a divisive model. Simultaneously, when operating with concentration values in very different ranges (i.e. large subtractive difference), proportional normalization via a divisive model can lead to more comparable values. For instance, the subtractive difference between a 200 mM sucrose patch and a 100 mM context is numerically large (100 mM), whereas the divisive difference (1.5x) maintains proportional comparability. Due to its mechanism of comparison, a subtractive model may remain equally sensitive (or insensitive) across concentration ranges. It potentially undervalues meaningful proportional differences at lower concentrations, while overvaluing large differences at high concentrations.

Ultimately, understanding the model with which *Drosophila* compare and integrate gustatory information would help create a framework to guide future examination of the neural mechanisms underlying decision-making.

1.6 Neural Mechanisms of Decision-Making in *Drosophila*

The ultimate purpose of developing behavioral models of decision-making in *Drosophila* is to identify the neural pathways and mechanisms underlying these behaviors. Relevant literature suggests RL models of behavior are mediated by dopaminergic neurons within the Mushroom Body, *Drosophila*'s primary structure responsible for learning, sensory integration, and memory.[8] [21] While specific cellular-level neural mechanisms for decision-making processes of *Drosophila* foraging are not known, optogenetic techniques used to identify neurons that play a role in such processes would make it possible to propose mechanisms. Dopaminergic Protocerebral Anterior Medial (PAM) neurons are of particular interest in this regard — these neurons project to lobes of the Mushroom Body, where they release dopamine signals which subsequently modulate Kenyon cell (KC) — Mushroom Body Output Neuron (MBON) synapses.[25] KCs process and encode sensory input that is transmitted to MBONs through the synapses that are modulated by PAM activity.[26] Thus, it is possible that PAM Neurons are implicated in the modulation of decision-making behaviors. This has been suggested by research

demonstrating that constitutive activation of PAM neurons disrupts the calculation of reward expectation, a core component of the RL model of decision-making.[27]

1.7 Aims and Hypotheses

To accomplish this project's objective of examining behavioral and neural mechanisms of decision-making in *Drosophila*, this project encompasses three primary aims.

Aim 1

How do *Drosophila* remember gustatory stimuli to inform behavioral decision-making? Multiple computational models have been proposed for decision-making in foraging, including the aforementioned four models: **input retention model, range retention model, Reinforcement Learning-based model, and Drift-Diffusion-based model.**

Considering the potential advantages and drawbacks of each model, as well as data from previous literature, we propose that the memory component of decision-making in *Drosophila* can be modeled using a Reinforcement Learning-based paradigm. As described above, past research used a Reinforcement Learning-based model to understand murine behavior in a similar food-choice assay, suggesting that a similar behavioral model could also be applied to *Drosophila*.[18]

Aim 2

How do *Drosophila* integrate gustatory stimuli to inform decision-making? Because the literature suggests that decision-making is based on the computation of a "relative value" related to the memory-based context, we propose two frameworks of how this comparative value may be computed — the aforementioned **divisive** and **subtractive models**. As previously illustrated, a **divisive** model may better inform behavior in a dynamic foraging environment representative of *Drosophila*'s natural environment — with both large differences at high sucrose concentrations and small differences at low concentrations. Thus, we hypothesize that the computation of relative value will follow a **divisive** model.

Aim 3

Lastly, are PAM dopaminergic neurons involved in the neural mechanisms underlying decision-making behaviors in *Drosophila*? Previous studies have implicated PAM Neurons in a Reinforcement Learning-model of decision-making, using constitutive activation to disrupt normal behavior. In this project, we also aim to explore the role of PAM Neurons in decision-making, but through optogenetic silencing of these neurons. Furthermore, we hope to connect this aim to Aim 1 and elucidate how PAM neurons might implement certain models of behavior, even if it is not the RL-based model suggested by previous literature. Ultimately, we hypothesize that PAM neuron silencing will result in a lack of context-dependent preference for "sweeter" gustatory stimuli, thus implicating PAM neurons in the neural mechanisms of decision-making.

Materials and Methods

This project contained two main experimental approaches. One approach assessed behavior in wildtype 2U *Drosophila* when presented with sucrose patches of varying concentrations; the other assessed behavior after optogenetic silencing of PAM reward neurons in genetically modified *Drosophila* when presented with sucrose patches of varying concentrations.

2.1 Fly Stocks and Husbandry

All flies were raised at 25° on cornmeal-molasses food. Experiments were performed on 2 to 5-day-old mated females. Additionally, all flies were food-deprived with water for 24 hours before behavioral assays. Flies used in optogenetic experiments were maintained in darkness, in order to prevent inadvertent activation of optogenetic pathways by ambient light. For optogenetic experiments, three days prior to behavioral assays, flies were switched from normal food to food containing 1 mM all trans-retinal. All-trans-retinal is an important cofactor necessary for the activation of the anion channelrhodopsin used for optogenetic silencing.[28] After two days of feeding on all trans-retinal containing food, optogenetic flies were food-deprived with an aqueous 1 mM trans-retinal solution for 24 hours before behavioral assays.

2.2 Genetic Crosses for Optogenetics

We used the Gal4-UAS system in *Drosophila* melanogaster to optogenetically silence PAM neurons with green light. Gal4, a transcriptional activator, is only transcribed in the neurons of interest (PAM neurons) when expressed using the transgene 58E02-Gal4.[26] When combined with UAS-GTACR1, Gal4 binds to an upstream activation sequence (UAS) that initiates transcription of the gene encoding GTACR1, a green light-sensitive anion channelrhodopsin. When stimulated by 525nm green light, GTACR1 allows an influx of anions to the interior of the cell membrane, hyperpolarizing and thereby inhibiting the cells in which it is activated.[29] Thus overall, by using a 58E02-Gal4 x UAS-GTACR1 cross, we can generate progeny in which we can inhibit PAM neuron activity via exposure to 525nm green light. In this experimental cross, UAS-GTACR1 virgin females are crossed with 58E02-Gal4 males. In addition to the

experimental group, we developed a control group by crossing 2U wild-type females with 58E02-Gal4 males, resulting in progeny that do not express GTACR1.

2.3 Preparation of Sucrose Solutions for Patches

All sucrose solutions were prepared by dissolving 1% agarose and a calculated amount of sucrose in water to create a given concentration of sucrose — 25 mM, 50 mM, 75 mM, 100 mM or 150 mM. 0 mM sucrose solutions were created with 1% agarose per volume water, but no sucrose. Milli-Q highly purified water was used to create all solutions. Solutions were stored at 4˚C and remade at minimum around once every two months to ensure freshness.

2.4 Behavioral Assays

For all behavioral assays, we heated sucrose-agarose solutions to 120˚C to liquefy them for pipetting We plated eighteen sucrose patches per fly "arena" in equidistant to one another and in a concentric manner within the circular arena. (Figure 4)

In order to test Aims 2 and 3, we used "regular choice" assays, following a one-to-one ratio of food types, where sucrose patches of concentration A alternated with patches of concentration B (Figure 4). In total, there were nine patches of concentration A and nine patches of concentration B. For Aim 2, we conducted regular choice behavioral assays in 75 mM vs. 25 mM, 150 mM vs. 100 mM, and 150 mM vs. 50 mM sucrose conditions, as well as non-choice (NC) controls for each of the aforementioned concentrations (25 NC, 50 NC, 75 NC, 100 NC, 150 NC). For optogenetic experiments in Aim 3, we conducted non-skewed behavioral assays in 50 mM vs. 25 mM sucrose conditions, as well as 50 mM and 25 mM NC controls.

In order to test Aim 1, we used "skewed choice" assays, where there were two patches of concentration A for every one patch of concentration B. (Figure 4) We conducted these skewed behavioral assays in 50 mM vs. 25 mM (2:1 and 1:2) sucrose conditions, as well as in 50 mM and 25 mM non-choice controls.

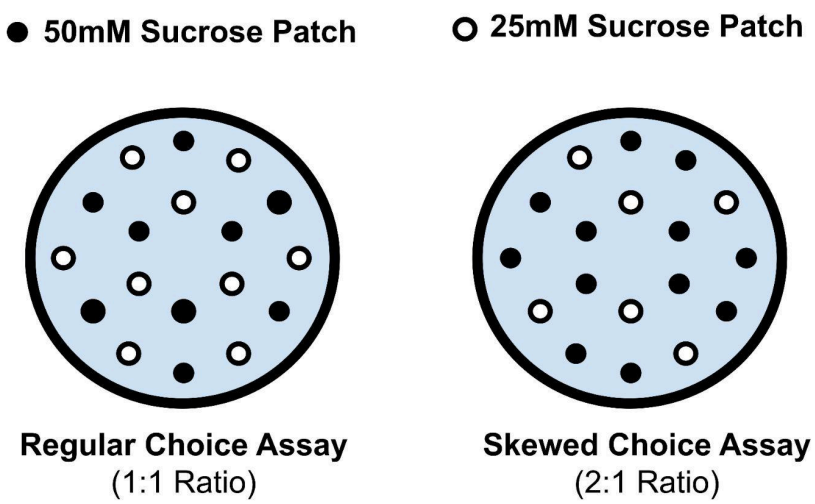

Figure 4. Sucrose patch plating scheme for "regular choice" and "skewed choice" assays
In **regular choice assays**, two alternating concentrations of sucrose patches are plated in an equidistant manner along two concentric circles within the circular arena. This ensures there is no location bias for any given concentration. **Skewed choice assays** also aim to eliminate location bias by selecting the same locations for patch plating. However, different concentrations are plated in these locations, due to the fact that the overall distribution is skewed towards one concentration (in this case, 50 mM).

The experimental setup (Figure 5) contained an overhead video camera positioned to record fly behavior in four arenas simultaneously. We limited potentially confounding variables for fly behavior by conducting video recordings in an enclosed environment to limit ambient light, with little or no ambient noise. Aim 1 and Aim 2 experiments recorded fly behavior in arenas for a 30-minute interval. Optogenetic experiments (Aim 3) recorded fly behavior for a 30-minute interval, but only 20 minutes were used to generate data, due to behavioral complications as detailed in results. Furthermore, for optogenetic experiments, we programmed light-emitting diodes (LEDs) to emit 525 nm green light were controlled by an Arduino and located 6cm beneath the platform supporting behavior arenas. We chose 525 nm green light in order to activate GTACR1 channels expressed in PAM neurons. Additionally, we used fans on either end of the platform to limit the thermal effects of intense LED light.

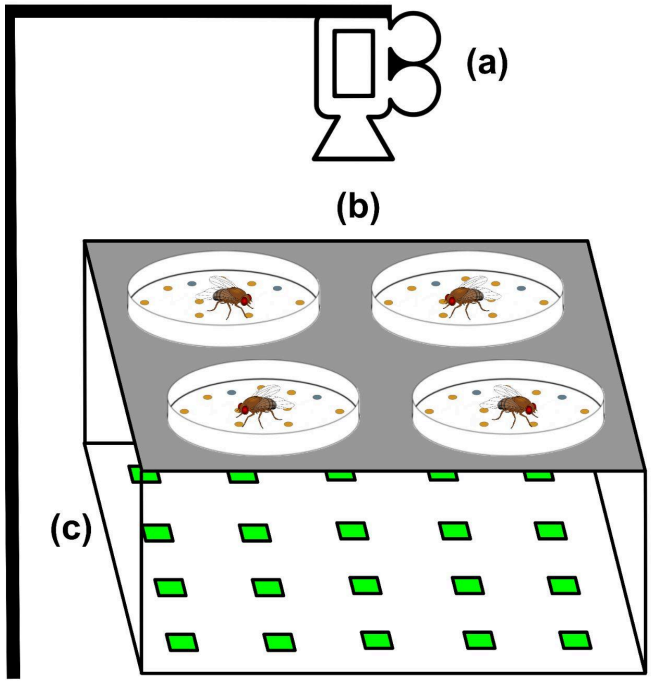

Figure 5. Experimental setup for behavioral assays.
Includes (a) suspended overhead video camera, (b) behavioral assays with *Drosophila* subjects, and (c) Arduino board with 525 nm green LEDs, used for optogenetics only.

2.5 Data Processing and Statistical Analysis

We initially processed all raw videos by removing the video background, so that each frame contained only the *Drosophila* subject. This allows for accurate tracking of the fly. We also recorded the locations of all sucrose patches within arenas using an X-Y coordinate system. We used ToxTrac software to track the fly's X-Y position at each frame. These outputs were further processed via custom Python code, in order to identify when each fly was on a patch, calculate the fly's trajectory through the arena, and extract other behavioral parameters. In this project, we chose to analyze median visit duration per fly, where visits are considered encounters with sucrose patches that last longer than 0.7s. We developed 0.7s as a threshold based on the minimum time it takes a fly to complete a feeding "burst," which is indicative of feeding behavior.[30] In addition to visit duration, we also considered "trajectory fit," which measures the similarity of a fly's path through a sucrose patch to a straight line. This is another quantification

of how flies interact with individual sucrose patches, beyond how long they spend on them. See Figure 5 for a detailed explanation of trajectory fit calculation and interpretation.

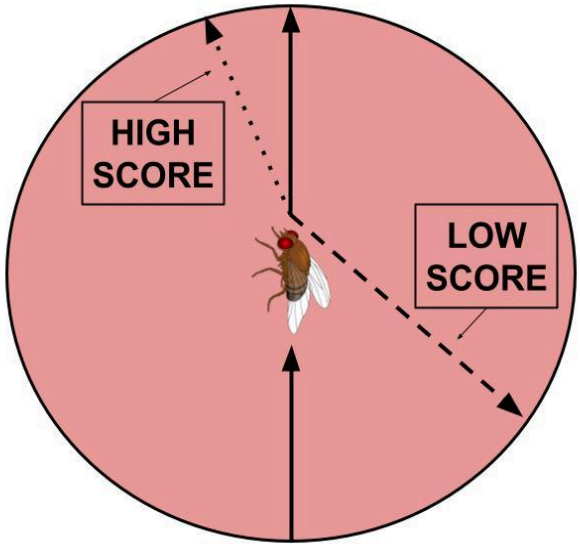

Figure 6. Schematic of Trajectory Fit for a Sucrose Patch
Trajectory Fit quantifies the similarity of a fly's path through a sucrose patch to a straight line. Our previous unpublished results show that when flies encounter a low-concentration sucrose patch, they tend to show a straighter path through the patch. This could be due to reduced feeding on the patch, which usually causes flies to make rotational movements during patch visits.[31] Conversely, interactions with high-concentration patches cause flies to take a path less similar to a straight line. These associations have been demonstrated by unpublished data from our lab (Peña Garcia, Unpublished). A high trajectory fit represents high similarity to a straight line, whereas a low score represents the opposite. Thus, a lower trajectory fit may be associated with more attractive gustatory stimuli.

We performed statistical analyses using Microsoft Excel 2021 and generated graphs using GraphPad Prism. We used Mann-Whitney U-Tests to compare between groups, given the non-parametric nature of the data. $P \leq 0.05$ is set as the threshold for significance. Error bars represent 1.5 times the interquartile range.

2.6 Resource Table

Reagent Type (Species) or Resource	Designation	Reference/Source
Genetic Reagent, *D. melanogaster*	R58E02-Gal4	Liu et al. (2012)[26]
Genetic Reagent, *D. melanogaster*	UAS-GTACR1	Deere et al. (2022)[17]
Genetic Reagent, *D. melanogaster*	Wild-Type Control 2U (isoCJ1)	Dubnau et al. (2001)[32]
Chemical Compound, Drug	Sucrose	Sigma-Aldrich
Chemical Compound, Drug	Agarose	Sigma-Aldrich
Chemical Compound, Drug	All-trans-retinal	Sigma-Aldrich
Chemical Compound, Drug	MilliQ Water	Emory University
Hardware	Arduino LED Board	Arduino
Software: Programming	Python 3.12	Anaconda
Software: Analysis	Excel 2021	Microsoft
Software: Graphing	Prism 10	GraphPad

Results

3.1 [Aim 1] Modulation of *Drosophila* foraging in response to altered sucrose patch prevalence does not support any one model of memory-informed decision-making.

After *Drosophila* encounter a patch, the decision to stay and feed, or leave and explore, depends upon knowing the prevalence of other options, which in turn depends on how it remembers previous encounters. Therefore, to differentiate between our proposed models for how *Drosophila* memorize gustatory stimuli and subsequently adjust foraging decisions, we assessed *Drosophila* behavior in skewed-choice assays. Within these skewed-choice assays, we distributed patches in ratios of either 2-50 mM : 1-25 mM (skewed pro-50 mM) or 1-50 mM : 2-25 mM (skewed anti-50 mM), and extracted data for the median visit duration per fly.

If we consider only 50 mM patch visits, both of the proposed “limited-context” models, **input retention and range retention, predict that visit duration on 50 mM patches in a NC condition is equal to visit duration in both skewed conditions.** This reflects the idea that these models do not consider the relative prevalence of 50 mM and 25 mM patches. For instance, if a fly memorizes each encounter of a binary sucrose-choice assay — and only feeds if a given stimulus is “as sweet” or “sweeter” than most other encountered stimuli — it will feed equal amounts on 50 mM patches regardless of whether it *only* encounters 50 mM patches (50NC) or encounters both 50 mM and 25 mM patches (skewed assays).

In contrast, both the RL-based and DDM-based models do predict differences in median visit durations across all assays. However, distinguishing between these models hinges on the difference between skewed pro-50 mM and skewed anti-50 mM conditions. The RL-based model memorizes context as an "expected value"—a running average compared against newly encountered sucrose patches to inform foraging. When there are more 25 mM patches (skewed anti-50 mM), the running average is low (near 25 mM), and the RL model predicts longer median visit durations. Conversely, the DDM-based model anticipates longer median visit durations when there are more 50 mM patches (skewed pro-50 mM), where increased interaction with 50 mM patches allows the fly to establish a "learned value" closer to the actual patch value, resulting in longer visits.

If we consider 25 mM patch visits, RL-based and DDM-based models predict the opposite of their predictions for 50 mM patch visits. **RL-based model predicts that engagement on 25 mM patches should be *lower* in a skewed pro-50 mM condition** — the memorized "expected value" is higher due to the increased prevalence of 50 mM patches, so a fly should know to reject a 25 mM patch quicker. Similar to above, a **DDM-based model suggests the opposite: that engagement on 25 mM patches should be *higher* in a skewed pro-50 mM condition, and *lower* in an anti-50 mM condition.**

Ultimately, these hypotheses are all also predicated on the notion that *Drosophila* have a baseline preference for, and exhibit higher median visit duration on "sweeter" patches. Previous research from our lab demonstrates this preference in regular-choice assays (Peña Garcia, Unpublished). However, we thought it important to recreate this baseline preference in skewed-choice assays to reinforce the applicability of this conclusion to our subsequent experiments.

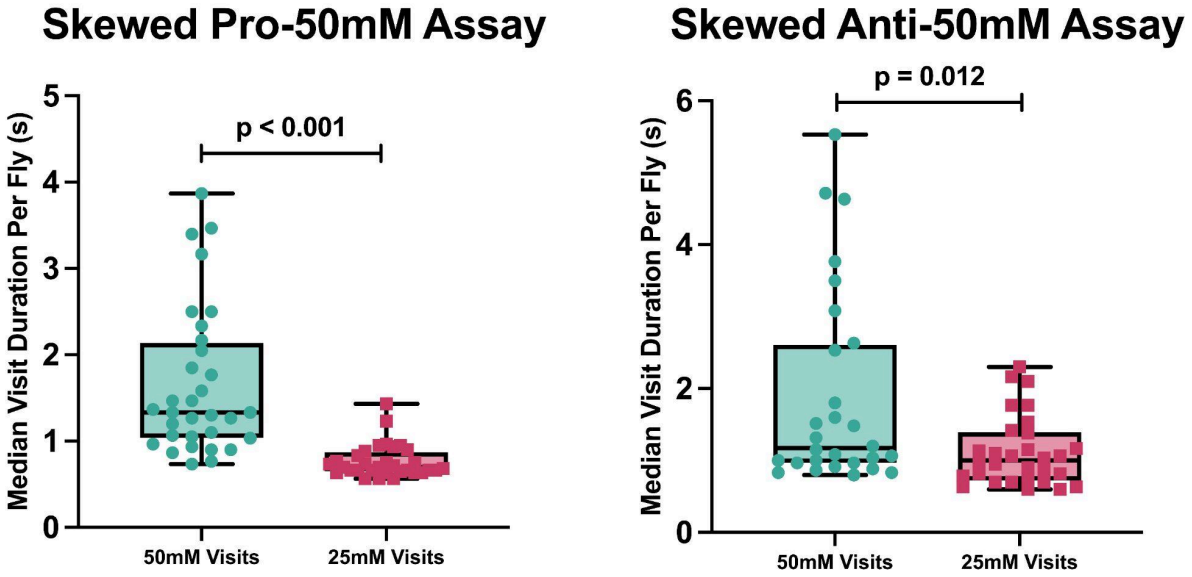

Figure 7. *Drosophila* spend more time on higher-concentration patches, regardless of the environmental context.

(a) Median visit duration per fly on 50 mM and 25 mM patches, in a skewed pro-50 mM assay. N=32 flies. Error bars represent 1.5 × IQR. P-values calculated using pairwise Mann-Whitney U-Tests.

(b) Median visit duration per fly on 50 mM and 25 mM patches, in a skewed anti-50 mM assay. N=29 flies. Error bars represent 1.5 × IQR. P-values calculated using pairwise Mann-Whitney U-Tests.

Indeed, our results suggest that *Drosophila* also exhibit a baseline preference for higher-concentration sucrose patches in skewed-choice assays (Figure 7). Next, comparing median visit duration among 50 mM patch visits, we observe significantly longer visit duration in both skewed assays, as compared to the 50NC assay (Figure 8a). According to our predictions for how input retention and range retention models inform behavior, this directly contradicts both models. Therefore, the data suggest that these "limited context" models may not be accurate in explaining stimulus memory in *Drosophila*. Yet, we do not observe a statistically significant difference between the two skewed choice assays — skewed pro-50 mM and skewed anti-50 mM.

We do, however, observe a statistically significant difference between the two skewed assays among 25 mM patch visits: visits are significantly shorter within the skewed pro-50 mM condition (Figure 8b). This data aligns with the predictions of the RL-based model. Thus, while the 25 mM patch data suggests the validity of an RL-based model over a DDM-based model, we cannot conclusively support an RL-based model, due to the non-significance observed in the 50 mM patch data. Still, the 50 mM patch data suggests that neither the input retention nor range retention models provide an adequate framework to model the role of memory in *Drosophila* decision-making.

However, visit duration is admittedly an imperfect proxy for active feeding behavior, and additional metrics may be useful to differentiate between models. Therefore, we also extracted and analyzed the "trajectory fit" metric from the behavioral data, which measures the path flies take across a patch, relative to a straight line. As previously outlined, in past preliminary data, we have shown that trajectory fit is inversely correlated to encounter duration (Peña Garcia, Unpublished). This is supported by literature, as rotational movements that may cause a fly to deviate from a straight-line path are associated with feeding-initiated local search behavior in *Drosophila*.[31] Feeding behavior and patch "engagement" increase on higher-concetration sucrose patches, thus these path deviations manifest as reduced trajectory fit scores on higher-concentration patches.

Because it predicts flies engage more with 50 mM sucrose patches in a skewed anti-50 mM assay, **an RL-based model also predicts that the average adjustment score will be closer to zero within this assay.** Furthermore, for 25 mM encounters, an **RL-based model predicts** the opposite — **that average trajectory fit is significantly higher in an anti-50 mM**

assay, as the flies will "know" to avoid these patches, and thus follow a straight path across the patch.

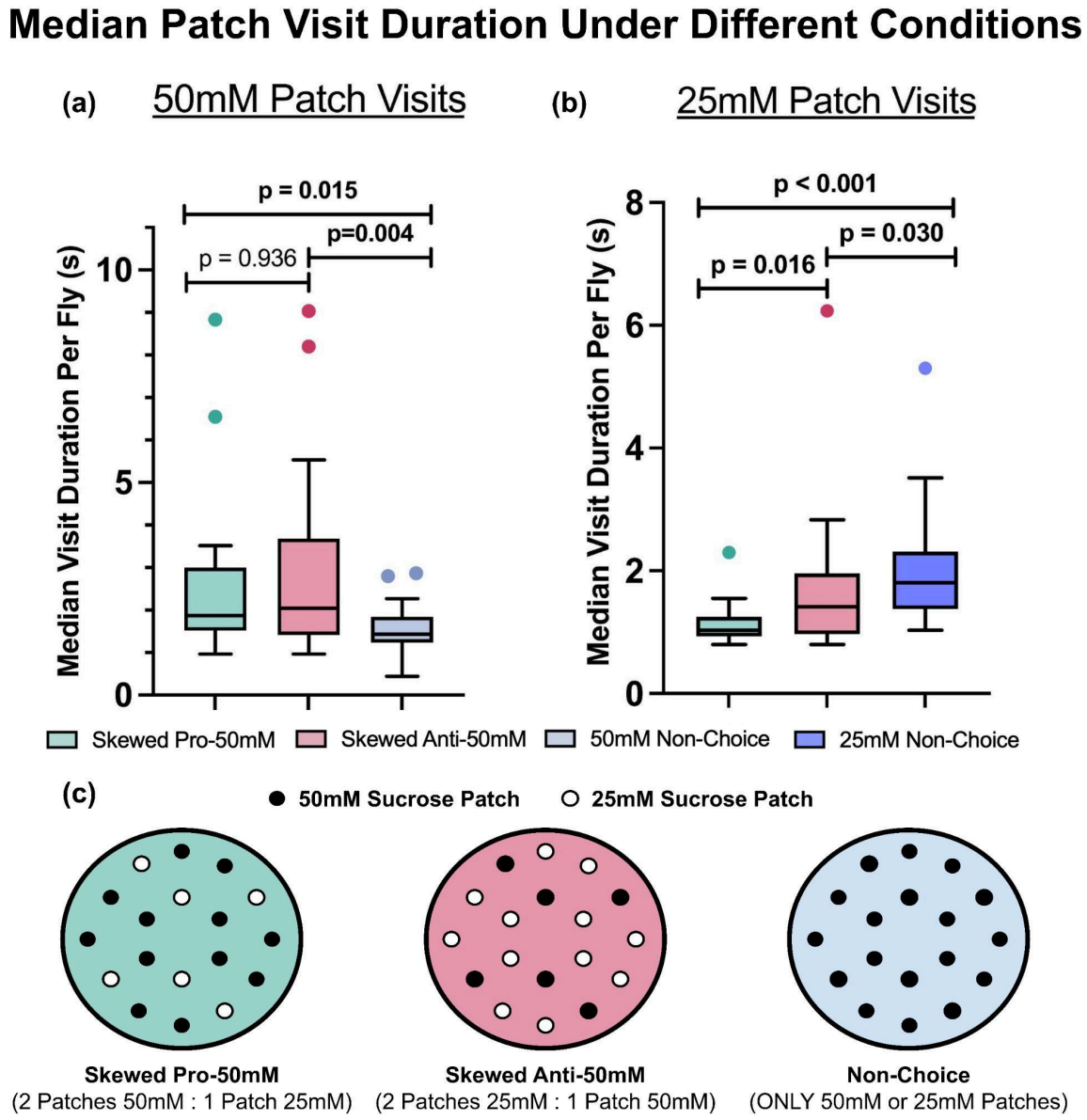

Figure 8. Median visit duration per fly under various skewed and non-choice assays.

(a) Median visit duration of 50 mM patches under skewed pro-50 mM (n=32 flies), skewed anti-50 mM (n=29), and 50 mM non-choice (n=32) conditions. Error bars are represented as $1.5 \times IQR$ above/below Q3 or Q1, respectively. P-values were calculated using pairwise Mann-Whitney U-tests.

(b) Median visit duration of 25 mM patches under skewed pro-50 mM (n=30), skewed anti-50 mM (n=29 flies), and 50 mM non-choice (n=36) conditions. Note: Outliers of 19.5 in pro-50mM and 13.5 in anti-50 mM groups are not shown in graph. Error bars and p-value calculations follow the same conventions as in Figure 6a.

(c) Schematic representation of skewed pro-50 mM, skewed anti-50 mM, and non-choice assays.

Among 50 mM patch encounters, average trajectory fit was significantly lower when 50 mM patches were less prevalent, in the anti-50 mM condition (Figure 9a). This lends support to a RL-based model of memory-informed decision-making as opposed to a DDM-based model because the data align with RL-based predictions. Additionally, we observed that average trajectory fit in the anti-50 mM condition was also significantly lower than in the 50 mM non-choice condition, further suggesting that "limited context" models may not be sufficient to explain the memorization process in *Drosophila*.

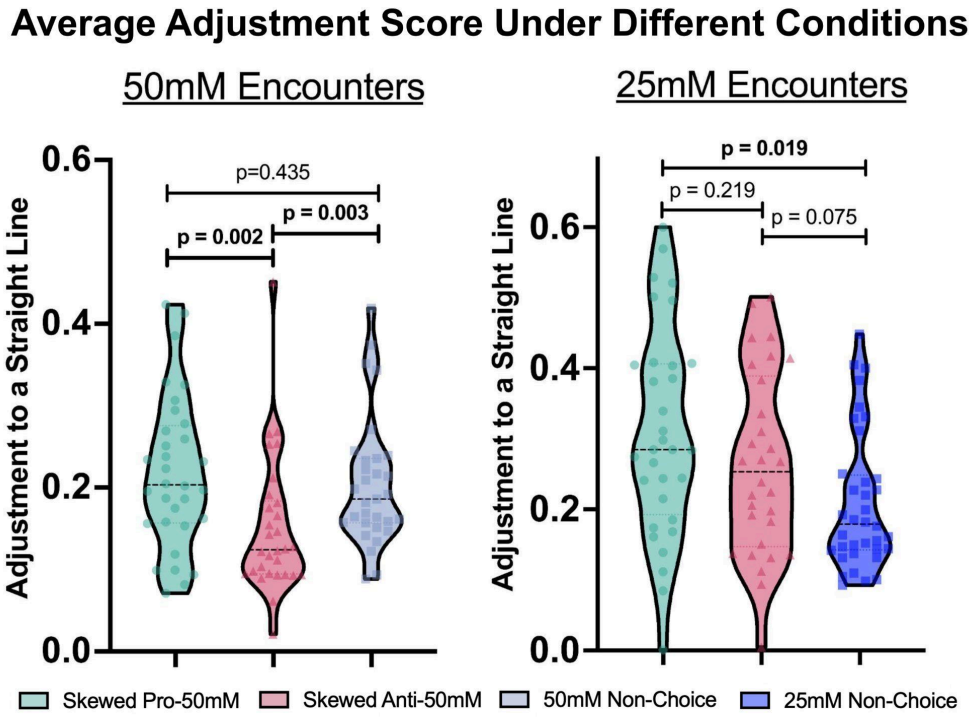

Figure 9. Average trajectory fit per fly under various skewed and non-choice assays.

(a) Average trajectory fit per fly for 50 mM encounters, i.e. adjustment to a straight line, under skewed pro-50 mM (n=32 flies), skewed anti-50 mM (n=30), and 50 mM non-choice (n=32) conditions. Data includes all patch encounters.

(b) Average trajectory fit per fly for 25 mM encounters, i.e. adjustment to a straight line, under skewed pro-50 mM (n=32 flies), skewed anti-50 mM (n=30), and 50 mM non-choice (n=36) conditions. Data includes all patch encounters.

Note: For both subfigures, encounters refer to any interaction with a patch — does not consider 0.7s threshold for visits. P-values were calculated using pairwise Mann-Whitney U-tests.

Unlike the 50 mM patch encounter data, our data within 25 mM patches does not directly follow the predictions of an RL-based model. The data trends in a direction that would support

the RL hypothesis, but no significant difference is observed between when 25 mM patches are more prevalent and when they are less prevalent (Figure 9b). It is, however, worth noting that among 25 mM encounters, the average trajectory fit was significantly higher when 25 mM patches were less prevalent (skewed pro-50 mM) than the 25NC condition, while there is no difference between when 25 mM patches were more prevalent (skewed anti-50 mM) and the 25NC condition. This may be construed as weak support for the RL-based model.

Ultimately, like the "visit duration" data, these data provide limited support for the RL-based model of memory-informed decision-making, but taken as a whole, we cannot say that these data conclusively suggest the validity of an RL-based model over a DDM-based model.

3.2 [Aim 2] In a two-choice assay, *Drosophila* use a divisive model of comparison to integrate stimuli and inform decision-making.

Having investigated models of memory-informed decision-making via the previous experiments, we next chose to examine how *Drosophila* compare and subsequently integrate gustatory stimuli. To study the validity of the two proposed models, a divisive model, and a subtractive model, we used a new series of regular-choice assays (1:1 ratio of sucrose patches), as well as their respective non-choice control groups. In order to study these models, we used three separate regular-choice assays of varying sucrose patch concentrations: 75 mM vs. 25 mM, 75 mM vs. 50 mM, and 150 mM vs. 100 mM.

In analysis of the collected data, we are specifically interested in how engagement on the higher sucrose concentration patches compares between the regular-choice assays. For example, we can examine how visit duration on 75 mM patches in a 75 mM vs. 25 mM assay compares to visit duration on 150 mM patches in a 150 mM vs. 100 mM assay. Here, it is important to understand that the *subtractive* difference of concentrations in both assays is equal — a 50 mM difference. However, the *divisive* difference of concentrations is not equal — a 3x and 1.5x difference, respectively. In this context, a **divisive model predicts that median visit duration is significantly higher in the 75 mM vs. 25 mM assay**, as compared to the 150 mM vs. 100 mM assay. Meanwhile, a **subtractive model predicts no difference in median visit duration.**

It is also important to compare visit duration on 75 mM patches in a 75 mM vs. 50 mM assay to visit duration on 150 mM patches in a 150 mM vs. 100 mM assay. Here, the divisive difference is equal, while the subtractive difference is not. Thus, a **subtractive model would**

predict a significant difference in visit duration, while a **divisive model would predict no difference.**

In our first comparison, the median visit duration of higher-concentration patches is significantly higher in the 75 mM vs. 25 mM assay, as compared to the 150 mM vs. 100 mM assay (Figure 10a). Furthermore, our second comparison shows no significant differences in higher-concentration patch visit duration, between the 75 mM vs. 50 mM and 150 mM vs. 100 mM assays (Figure 10b). As previously outlined, both of these predictions align precisely with the predictions of a divisive model of comparison. Thus, overall, our data provide support for the validity of this model.

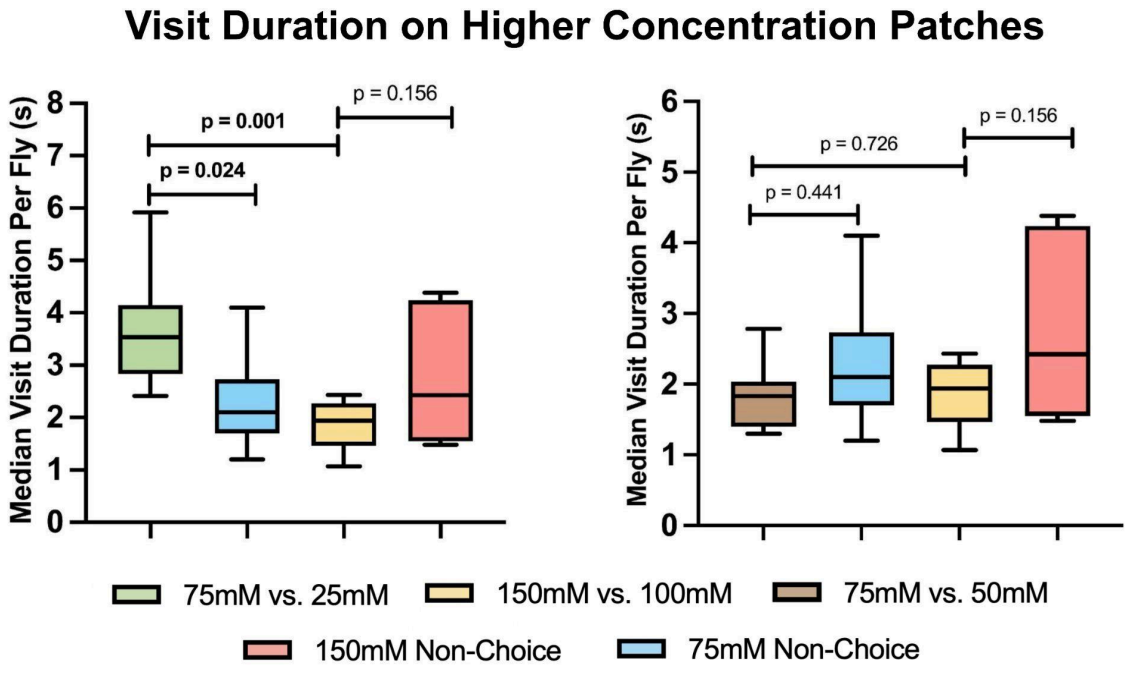

Figure 10. Modulations in sucrose patch engagement support a divisive model of comparison in *Drosophila*.

(a) Engagement, quantified as median duration visit per fly, is compared in four assays — 75 mM vs. 25 mM (n=8), 75NC (n=7), 150 mM vs. 100 mM (n=8), and 150 mM NC (n=8). Regular choice conditions share a subtractive difference in concentration, but have varying divisive differences.

(b) Engagement, quantified as median duration visit per fly, is compared in four assays — 75 mM vs. 50 mM (n=8), 75NC (n=7), 150 mM vs. 100 mM (n=8), and 150 mM NC (n=8). Regular choice conditions share a divisive difference in concentration, but have varying subtractive differences.

Note: For both subfigures — visits are defined as any interaction with a sucrose patch for greater than 0.7s. Error bars represented as 1.5 x IQR above/below Q3 or Q1, respectively. P-values were calculated using pairwise Mann-Whtitney U-Tests.

Furthermore, the data shown in Figure 10B exhibits no significant differences between either regular-choice assay and their respective non-choice controls. At first glance, this may appear to contradict literature that emphasizes the importance of relative values, created through the presence of alternative patch options, in decision-making.[24] However, it is also plausible that in order for alternative patch options to affect decision-making, a "threshold" of divisive difference must exist. That is, regular-choice assays of Figure 10B may not vary from the non-choice assays because the divisive difference — 1.5x — is too low. Thus, understanding whether such a "threshold" exists, and elucidating its value may be a compelling line of future inquiry.

3.3 [Aim 3] The role of Protocerebral Anterior Medial (PAM) neurons within the neural mechanisms of decision-making in *Drosophila* is unclear.

Our first aim found partial support for an RL-based model of memory-informed decision-making, and prior work identifies PAM neurons as key components in RL-driven neural mechanisms.[27] Thus, having explored the behavioral mechanisms of decision-making in our first two aims, we focused on examining PAM neurons as a potential component of the neural mechanism underlying decision-making in *Drosophila* foraging.

58E02-Gal4/UAS-GTACR1 flies contain an anion-conducting channelrhodopsin localized to dopaminergic PAM neurons. Thus, when activated by 525 nm green light, this channelrhodopsin silences PAM neuron activity by preventing depolarization. These PAM neuron-silenced flies were compared to 58E02-Gal4/+ controls in 50 mM vs. 25 mM and 100 mM vs. 50 mM assays. Thus, considering visits of only one given concentration, it stands to reason that median visit duration will be longer among controls if our hypothesis regarding PAM neurons' involvement in decision-making is correct. As with aim one, our first step was to determine whether a baseline preference for sweeter options exists in both controls and PAM neuron-silenced flies. While PAM neuron-silenced flies were hypothesized to lose context-dependent preference, they still should exhibit longer visit duration on high-concentration patches, as innate preference will likely mediate some portion of behavior.

Counterintuitively, only 58E02-Gal4/UAS-GTACR flies in the 50 mM vs. 25 mM assay demonstrate this baseline preference in our data (Figure 11a). This unexpected result renders it impossible to draw conclusions from comparisons across visits of one concentration, so they were not included for intepretation. Ultimately, we are unable to make any substantive claims about the role of PAM neurons in the neural mechanisms of decision-making.

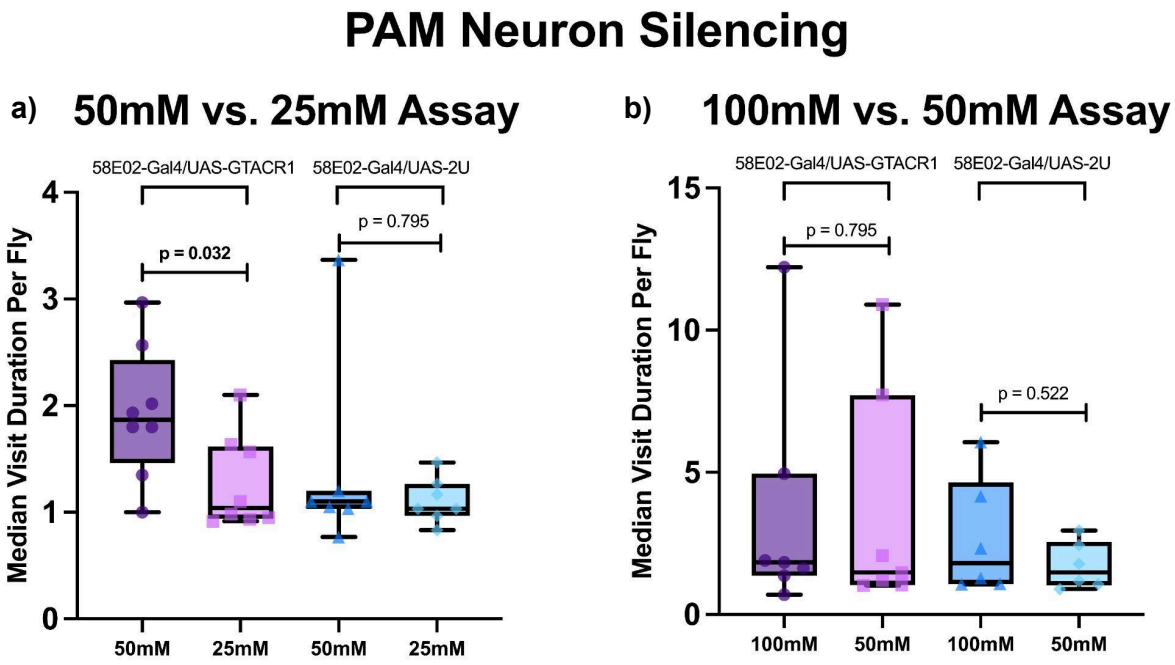

Figure 11. *Drosophila* do not demonstrate a baseline preference for high-concentration surcrose patches across optogenetically manipulated experimental and control groups.

(a) Graph shows median visit duration on both 50 mM and 25 mM sucrose patches in 58E02-Gal4/UAS-GTACR (n=8) flies and 58E02-Gal4/+ (n=7) control flies. PAM neurons are silenced in 58E02-Gal4/UAS-GTACR flies.

(b) Graph shows median visit duration on both 100 mM and 50 mM sucrose patches in 58E02-Gal4/UAS-GTACR (n=8) flies and 58E02-Gal4/+ (n=7) control flies. PAM neurons are silenced in 58E02-Gal4/UAS-GTACR flies.

Note: Fly behavior was recorded over a twenty, rather than thirty-minute period. For both assays, visits are defined as sucrose patch encounters greater than 0.7s. Error bars represent minimum and maximum visit duration values per group. Significance was analyzed via pairwise Mann-Whitney U Tests.

That said, there are multiple limitations of the above data. Firstly, while the sample size (seven or eight flies per condition) is appropriate for a pilot study, in order to gain a more robust

understanding of the statistical differences between groups, a larger sample size is needed. Even more importantly, fly behavior may have been impacted by heat conducted from the green-light LEDs to the fly behavior arenas (see discussion for further qualification). As a result of these limitations, there is a strong possibility that these data may be inconclusive.

Discussion

4.1 Discussion of Results

In this project, we sought to investigate the behavioral and neural mechanisms of decision-making in *Drosophila* during sucrose patch foraging tasks. Specifically, we investigated (1) how *Drosophila* use memory of gustatory stimuli to inform decision-making, (2) how they compare these stimuli, and (3) whether dopaminergic PAM neurons are implicated in the neural mechanisms underlying behavior within the studied sucrose patch foraging tasks.

To investigate the behavioral mechanisms of decision-making in Aim 1, we proposed four potential models for memory-informed decision-making rooted in previous literature. These included two "limited context models" — input retention and range retention — as well as a Reinforcement Learning-based and Drift Diffusion-based model. Overall, we found partial support for a RL-based model of memory-informed decision-making. Data for median visit duration on 25 mM sucrose patches closely follow the predictions of an RL-based model across all three assays, as does data for trajectory fit on 50 mM sucrose. That said, taken in their entirety, the data do not comprehensively support any one specific model. This is due to the trends in visit duration among 50 mM sucrose patches, and the trends in trajectory fit on 25 mM patches. Additional inquiries may be useful in better distinguishing the Reinforcement Learning-based models from other models of memory-informed decision-making.

Nonetheless, the partial support demonstrated for an RL-based model is significant on multiple levels. If memory in *Drosophila* were governed by an RL-based model, this would suggest that flies use expected-value-based decision strategies — as literature supports is present in many other complex organisms, including humans.[33] This result supports the idea that key elements of decision-making are evolutionarily conserved, which could possibly allow for neural findings collected using the *Drosophila* connectome to be investigated and applied to more complex animal models. Furthermore, since previous research implicates PAM neurons in RL-based decision-making processes, behavioral alignment with an RL-based model would lend functional support for PAM neuron involvement in the neural circuitry thought to underlie memory-informed decision-making. This could help bridge the gap between behavior and the cellular-level mechanisms driving it.

In Aim 2, the data holistically support a divisive model of comparison and subsequent integration. Ultimately, this result underlines the importance of the advantages conferred by a divisive model, as previously discussed. A productive line of future research could explore how neural mechanisms in the *Drosophila* brain support this computation — it is possible that the activity of various second-order neurons known to integrate gustatory stimuli is proportional to divisive differences in these stimuli.[34] This conclusion is also important for the research design and hypotheses of future food-choice experiments in *Drosophila*. Hypotheses and design principles should consider that the degree of preference for higher-concentration food patches is dependent on the concentration ratios for options presented.

Additionally, no significant difference in median visit duration was observed between the assays conducted in Figure 10b. It is possible that these data are a false negative result due to the small sample size (seven or eight flies per condition), and they missed a significant difference that should have been present. However, assuming the validity of this data, this experiment suggests a

certain divisive threshold must be surpassed for the comparison of options to affect decision-making behavior. Because 75 mM vs. 50 mM and 150 mM vs. 100 mM assays — divisive differences of 1.5x — yielded no differences in visit duration from 75NC and 150NC assays — divisive differences of 1x — it stands to reason that this potential threshold must be at least a 1.5x difference.

Our third line of inquiry, focusing on dopaminergic PAM neurons, provided counterintuitive yet inconclusive results. We were unable to observe a significant baseline preference for high-concentration sucrose patches across the conditions tested. This lack of signficance would conflate any further comparisons made between groups, thus we are unable to interpret the behavior of the PAM neuron-silenced flies. However, the limitations of this study put these unusual results into context. As previously outlined, arenas conducted residual heat from the green-light LEDs, raising arena surface temperatures by as much as 10˚C. This likely impacted fly behavior, despite the installation of electronic fans to provide convection cooling. Furthermore, while only twenty minutes of each behavioral video was analyzed to compensate for irregular movement, this may have impacted our interpretation of the data.

4.2 Future Directions

The limitations inherent in our PAM-silencing experiment also create immediate possible lines of future experimentation. Alternative means of cooling, reduced green-light intensity, and constitutive activation of PAM neuron-localized channelrhodopsins are all possible methods of working around this undesirable heat effect. Additionally, experiments in both Aim 2 and Aim 3 may benefit from continued data collection involving larger sample sizes. This, in turn, could generate more robust statistical conclusions. A second major limitation of this project is the use of various metrics — including visit duration and adjustment score — as imperfect proxies for feeding. The only apparent method to remedy these imperfect comparison metrics is using a computationally intensive method, which involves labeling proboscis extension (a more direct measure of feeding) frame by frame. Our lab has begun the foundational work necessary to implement such a method.

The primary line of future questioning following this project should broadly concern cellular-level neural mechanisms underlying decision-making in *Drosophila*, as the availability of the connectome offers us the potential to elucidate a precise neural circuit responsible for these behaviors.[6] [7] This may include examining the effect of silencing PPL1 neurons or other dopaminergic neurons innervating the Mushroom Body, given that past research implicates such dopaminergic neurons in modulating the valence of gustatory stimuli.[35] Alternatively, Kenyon Cells are also an intriguing target for neuromanipulation, as they are the primary neurons responsible for encoding and processing sensory input within the Mushroom Body.[36] Nonetheless, our findings within this project lay the groundwork for further exploration into the behavioral mechanisms and cell-level circuitry of value-based decision-making behaviors in *Drosophila*, a high-potential model system.

References

1. Rahman S, Sahakian BJ, Cardinal RN, Rogers RD, Robbins TW. Decision making and neuropsychiatry. Trends in Cognitive Sciences. 2001 Jun 1;5(6):271–7. doi:10.1016/s1364-6613(00)01650-8

2. Krmpotich T, Mikulich-Gilbertson S, Sakai J, Thompson L, Banich MT, Tanabe J. Impaired decision-making, higher impulsivity, and drug severity in substance dependence and pathological gambling. Journal of Addiction Medicine. 2015;9(4):273–80. doi:10.1097/adm.0000000000000129

3. Blouzard E, Pouchon A, Polosan M, Bastin J, Dondé C. Effort-Cost Decision-Making Among Individuals With Schizophrenia. JAMA Psychiatry. 2023 Jun 1;80(6):548. doi:10.1001/jamapsychiatry.2023.0553

4. Attaallah B, Toniolo S, Maio MR, Husain M. Apathy and effort‐based decision‐making in Alzheimer's disease and subjective cognitive impairment. Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring. 2024 Oct 16;16(4). doi:10.1002/dad2.70013

5. Euston DR, Gruber AJ, McNaughton BL. The role of medial prefrontal cortex in memory and decision making. Neuron. 2012 Dec 20;76(6):1057–70. doi:10.1016/j.neuron.2012.12.002

6. Schlegel P, Yin Y, Bates AS, Dorkenwald S, Eichler K, Brooks P, et al. Whole-brain annotation and multi-connectome cell typing of Drosophila. Nature. 2024 Oct 2;634(8032):139–52. doi:10.1038/s41586-024-07686-5

7. Dorkenwald S, Matsliah A, Sterling AR, Schlegel P, Yu S, McKellar CE, et al. Neuronal wiring diagram of an adult brain. Nature. 2024 Oct 2;634(8032):124–38. doi:10.1038/s41586-024-07558-y

8. Modi MN, Shuai Y, Turner GC. The Drosophila Mushroom Body: From Architecture to Algorithm in a Learning Circuit. Annual Review of Neuroscience. 2020 Jul 8;43(1):465–84. doi:10.1146/annurev-neuro-080317-0621333

9. Mahishi D, Huetteroth W. The prandial process in flies. Current Opinion in Insect Science. 2019 Nov 6;36:157–66. doi:10.1016/j.cois.2019.09.004

10. Jefferis GSXE, Luo L. The Development of the Olfactory System. Comprehensive Molecular Insect Science. 2005 May 28;421–63. doi:10.1016/b0-44-451924-6/00007-7

11. Montell C. A taste of the Drosophila gustatory receptors. Current Opinion in Neurobiology. 2009 Aug 5;19(4):345–53. doi:10.1016/j.conb.2009.07.001

12. Kolling N, Akam T. (Reinforcement?) Learning to forage optimally. Current Opinion in Neurobiology. 2017 Oct 15;46:162–9. doi:10.1016/j.conb.2017.08.008

13. Liman ER, Zhang YV, Montell C. Peripheral coding of taste. Neuron. 2014 Mar 5;81(5):984–1000. doi:10.1016/j.neuron.2014.02.022

14. Marella S, Mann K, Scott K. Dopaminergic Modulation of Sucrose Acceptance Behavior in Drosophila. Neuron. 2012 Mar 8;73(5):941–50. doi:10.1016/j.neuron.2011.12.032

15. Seidenbecher SE, Sanders JI, von Philipsborn AC, Kvitsiani D. Reward foraging task and model-based analysis reveal how fruit flies learn value of available options. PLOS ONE. 2020 Oct 2;15(10). doi:10.1371/journal.pone.0239616

16. Kim IS, Dickinson MH. Idiothetic Path Integration in the Fruit Fly Drosophila melanogaster. Current Biology. 2017 Aug 7;27(15). doi:10.1016/j.cub.2017.06.026

17. Deere JU, Devineni AV. Taste cues elicit prolonged modulation of feeding behavior in Drosophila. iScience. 2022 Sept 17;25(10):105159. doi:10.1016/j.isci.2022.105159

18. Verharen JP, de Jong JW, Zhu Y, Lammel S. A computational analysis of mouse behavior in the sucrose preference test. Nature Communications. 2023 Apr 27;14(1). doi:10.1038/s41467-023-38028-0

19. Davidson JD, El Hady A. Foraging as an evidence accumulation process. PLOS Computational Biology. 2019 Jul 24;15(7). doi:10.1371/journal.pcbi.1007060

20. Wang X-J. Decision Making in Recurrent Neuronal Circuits. Neuron. 2008 Oct 23;60(2):215–34. doi:10.1016/j.neuron.2008.09.034

21. Bennett JE, Philippides A, Nowotny T. Learning with reinforcement prediction errors in a model of the Drosophila mushroom body. Nature Communications. 2021 May 7;12(1). doi:10.1038/s41467-021-22592-4

22. Lyutova R, Selcho M, Pfeuffer M, Segebarth D, Habenstein J, Rohwedder A, et al. Reward signaling in a recurrent circuit of dopaminergic neurons and peptidergic Kenyon cells. Nature Communications. 2019 Jul 15;10(1). doi:10.1038/s41467-019-11092-1

23. Eschbach C, Fushiki A, Winding M, Afonso B, Andrade IV, Cocanougher BT, et al. Circuits for integrating learned and innate valences in the insect brain. eLife. 2021 Nov 10;10. doi:10.7554/elife.62567

24. Hunter LE, Daw ND. Context-sensitive valuation and learning. Current Opinion in Behavioral Sciences. 2021 Jun 9;41:122–7. doi:10.1016/j.cobeha.2021.05.001

25. Cohn R, Morantte I, Ruta V. Coordinated and Compartmentalized Neuromodulation Shapes Sensory Processing in Drosophila. Cell. 2015 Dec 17;163(7):1742–55. doi:10.1016/j.cell.2015.11.019

26. Liu C, Plaçais P-Y, Yamagata N, Pfeiffer BD, Aso Y, Friedrich AB, et al. A subset of dopamine neurons signals reward for odour memory in Drosophila. Nature. 2012 Jul 18;488(7412):512–6. doi:10.1038/nature11304

27. Rajagopalan AE, Darshan R, Hibbard KL, Fitzgerald JE, Turner GC. Reward expectations direct learning and drive operant matching in Drosophila. Proceedings of the National Academy of Sciences. 2023 Sept 21;120(39). doi:10.1073/pnas.2221415120

28. Li H, Huang C-Y, Govorunova EG, Schafer CT, Sineshchekov OA, Wang M, et al. Crystal structure of a natural light-gated anion channelrhodopsin. eLife. 2019 Jan 7;8. doi:10.7554/elife.41741

29. Mohammad F, Stewart JC, Ott S, Chlebikova K, Chua JY, Koh T-W, et al. Optogenetic inhibition of behavior with anion channelrhodopsins. Nature Methods. 2017 Jan 23;14(3):271–4. doi:10.1038/nmeth.4148

30. Itskov PM, Moreira J-M, Vinnik E, Lopes G, Safarik S, Dickinson MH, et al. Automated Monitoring and quantitative analysis of feeding behaviour in Drosophila. Nature Communications. 2014 Aug 4;5(1). doi:10.1038/ncomms5560

31. Murata S, Brockmann A, Tanimura T. Pharyngeal stimulation with sugar triggers local searching behavior in Drosophila. Journal of Experimental Biology. 2017 Jan 1; doi:10.1242/jeb.161646

32. Dubnau J, Grady L, Kitamoto T, Tully T. Disruption of neurotransmission in Drosophila mushroom body blocks retrieval but not acquisition of memory. Nature. 2001 May 24;411(6836):476–80. doi:10.1038/35078077

33. Lee D, Seo H, Jung MW. Neural basis of reinforcement learning and decision making. Annual Review of Neuroscience. 2012 Jul 21;35(1):287–308. doi:10.1146/annurev-neuro-062111-150512

34. Miyazaki T, Lin T-Y, Ito K, Lee C-H, Stopfer M. A gustatory second-order neuron that connects sucrose-sensitive primary neurons and a distinct region of the gnathal ganglion in the Drosophila brain. Journal of Neurogenetics. 2015 Jul 3;29(2–3):144–55. doi:10.3109/01677063.2015.1054993

35. Mao Z, Davis RL. Eight Different Types of Dopaminergic Neurons Innervate the Drosophila Mushroom Body Neuropil: Anatomical and Physiological Heterogeneity. Frontiers in Neural Circuits. 2009 Jun 30;3. doi:10.3389/neuro.04.005.2009

36. Kirkhart C, Scott K. Gustatory learning and processing in the Drosophila mushroom bodies. The Journal of Neuroscience. 2015 Apr 15;35(15):5950–8. doi:10.1523/jneurosci.3930-14.2015