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The Role of Dopamine in Decision Making Processes in Drosophila Melanogaster

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The Role of Dopamine in Decision Making Processes in *Drosophila Melanogaster*

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Abstract

Understanding the neural processes that mediate decision making is a relatively new field of investigation in the scientific community. With the ultimate goal of understanding how humans decide between one path and another, simpler models such as *Drosophila Melanogaster*, the common fruit fly, are often utilized as a way of determining the neural circuits involved in these decision-making processes. One of the most important decisions flies make is the decision of where to lay their eggs (oviposit). Choosing the proper substrate upon which to lay eggs is a crucial decision that can ultimately impact their fecundity. This paper investigates the field of decision-making neuroscience research previously conducted in order to provide background information and point out the void that my research is attempting to fill. In conducting research, I first began by collecting data on the number of eggs laid by wildtype flies on each substrate type (sucrose, yeast, combination, or plain) within the 20 chamber two-choice preference assay. Following this, the same procedure was conducted using dopamine knockout flies created by crossing KIR2.1 genetically encoded flies with specific dopamine output neurons which inhibited their function. Our lab found that wildtype flies prefer yeast and avoid sucrose. They also tend to choose a plain substrate in Plain vs. Sucrose-Yeast. Though the genetically altered flies also prefer plain, a significant decrease in preference was observed in four of the mushroom body output neuron lines (057B, 027C, 542B, 543B) indicating that these lines may play a more significant role in determining this preference for Plain over Sucrose-yeast. These neurons that mediate crucial decisions for fruit flies can hopefully one day be correlated to the dopamine neurons in the human brain that help us make simple, everyday decisions and even life-changing decisions such as where to settle down someday and lay our own eggs.

The Role of Dopamine in Decision Making Processes in *Drosophila Melanogaster*

As humans, we make countless decisions every day. From simple everyday choices, such as what to have for breakfast or what shoes to wear, to more complicated life-changing decisions such as who to marry or where to settle down and start a family, these decisions make us who we are. Scientists estimate that the average person makes around 35,000 decisions per day (Daum, 2012). Accounting for an average of 7 hours of sleep per night where no decisions are made, this staggering number averages to roughly 30 decisions every single minute of our lives. Some of these decisions, such as a president declaring war, or a doctor deciding what surgical technique to implement in the Emergency Room, can have life-altering consequences and impact many lives. In addition, these one-time decisions are not the only ones that can change lives. The continual, day-in, day-out decisions that everyone must make can lead people with addictions down a very dark road. Having to decide between momentary pleasure and life-long health and wellbeing is a difficult decision for millions of people worldwide. For these reasons and many others just like them, understanding the neuroscience behind how decisions are made has become crucial to scientists hoping to better understand the process of why we do what we do.

Using *Drosophila Melanogaster* as a Research Model

In attempting to discover the intricacies of the neuronal factors that contribute to decisions, scientists realized that the human brain is far too complex and that this quest must begin with a simpler model. Not only are the intricacies of the human brain beyond what we are capable of presently studying, but running human trials can be costly, time consuming, and oftentimes unethical (Tolwinski, 2017). With these known limitations, a model had to be found by scientists that could provide a basis for the exploration of decision-making neuroscience. Luckily, a well-known model had already existed and been used for over one hundred years in biomedical science research: *Drosophila Melanogaster.* More commonly known as the fruit fly,

Drosophila Melanogaster has provided scientists with an inexpensive, rapidly regenerating, and a model genetically similar to humans for scientific research (Tolwinski, 2017). The original history as to how the fruit fly first came to be used as a model for scientific exploration remains a mystery, but the vast number of studies taking advantage of its usefulness overtime are certainly documented. Beginning with Thomas Hunt Morgan's chromosomal studies to Hermann Muller's exploration on the effects of X-rays on mutation rates, studies conducted with *Drosophila Melanogaster* earned many Nobel Prizes and expanded the horizon of biological knowledge (Tolwinski, 2017). A major turning point in *Drosophila* studies came when Seymour Benzer utilized this model as a way of exploring the effects on behavior that genes can play (Benzer, 1971). By exploring how certain genes can lead to the manifestation of specific phenotypes, Benzer paved the way for future studies on genetic diseases in humans. Continuing into present day, as technology became more advanced, such as through the invention of CRISPR/Cas9 knockout strategies, more fine-tuned experiments could be run that targeted specific genes and their subsequent proteins (Wangler et al., 2017). Knockout strategies enable gene tagging, inactivation, and overexpression of specific target genes which puts the power of controlling the experiment in the hands of the experimenter. Utilized in countless experiments for decades, *Drosophila Melanogaster* has provided a simple, inexpensive, and effective research model for advancing scientific knowledge. Though the translation to human-life improvement may be difficult to see at first, a slightly deeper look into the short history of this model allows one to see the vast discoveries the fruit fly has allowed scientists to make.

In more recent years, *Drosophila Melanogaster* has been distinctly used for expanding research in the field of neuroscience. Because the genome of the fruit fly is known and sequenced, with new technology like CRISPR, studying specific neural circuits has become

easier and more accessible. These neural circuits in the fruit fly can then be investigated for what innate behaviors they mediate. One of the earliest neuronal mechanisms explored using *Drosophila* was olfactory learning and memory. Beginning in the 1970's with William G. Quinn and colleagues' invention of a reliable assay to measure olfactory learning, experiments investigating the association of neuronal circuits and behavioral outputs expanded quickly (McGuire, Deshazer, & Davis, 2005). Once baseline experiments had been run exploring how wildtype fruit flies learn and recall conditioned stimuli, mutations to these neurons and the behavioral effects that ensue could be explored. Two methods were employed to explore mutations and their effects: chemical mutagenesis and brain structure mutations. While the Quinn lab explored chemical mutagenesis, a lab headed by Heisenberg and Boehl investigated the latter's effects. Exploring the brain structures required for learning and memory, a separate lab headed by Heisenberg and Boehl found that damage to the mushroom body, a main structure in the fruit fly brain that will be explain in detail later on, causes impairment in olfactory learning (McGuire, Deshazer, & Davis, 2005). These two distinct methods for investigating which genes and proteins play a role in learning and memory paved the way for subsequent labs to explore the effects of neuronal dysfunction on behavioral outputs.

Before delving into the neuroscience research behind the specific behavioral output of decision making, I wanted to provide some background on the structural anatomy of the mushroom bodies inside the brain of *Drosophila Melanogaster*. First discovered in 1850 by French Biologist Félix Dujardin, the structures of the Mushroom Body in *Drosophila* are so named because of their visual similarity to mushrooms (Strausfeld et al., 1998). After Dujardin's discovery, countless experiments were carried out by subsequent labs both confirming and furthering his hypothesis that the mushroom body played a role in intelligent behavior. It was not

until very recently in scientific history that suggestions of the possibility of the mushroom body's role in learning and memory arose. This discovery came about when experiments lesioning the mushroom bodies in *Formica rufa* led to their inability to navigate a maze using olfactory cues (Vowles, 1964). To understand the effect of these lesions, one must understand the anatomical structure of the mushroom bodies. The mushroom bodies are a pair of structures in the brain of the fruit fly that are composed of 2500 intrinsic neurons per brain hemisphere. These axon bundles, known as Kenyon cells, project toward the anterior face of the brain via a dense structure known as the peduncle. Here, in the unmyelinated regions known as the lobes or neuropil of the mushroom body, these axons synapse on the dendrites of follower neurons (See Figure 1) (McGuire, Deshazer, & Davis, 2005). These mushroom body neurons project to several different classes of output neurons that then project to diverse brain regions so studying the overall impact of legions to this area can be complicated. One way that scientists have been able to subdivide, and thus simplify, these regions has been through immunohistochemical examination of the expression of different genes expressed in the mushroom bodies (McGuire, Deshazer, & Davis, 2005). Upon examination, it has been shown that specific proteins localize to subsets of neurons and regions within those neurons, thus allowing for three distinct subsets of the mushroom body to be identified: α/β, α'/β', and γ (McGuire, Deshazer, & Davis, 2005). These subsets of neurons not only imply distinct structural differences between the regions, but more importantly, functional distinctions amongst them. Understanding the history

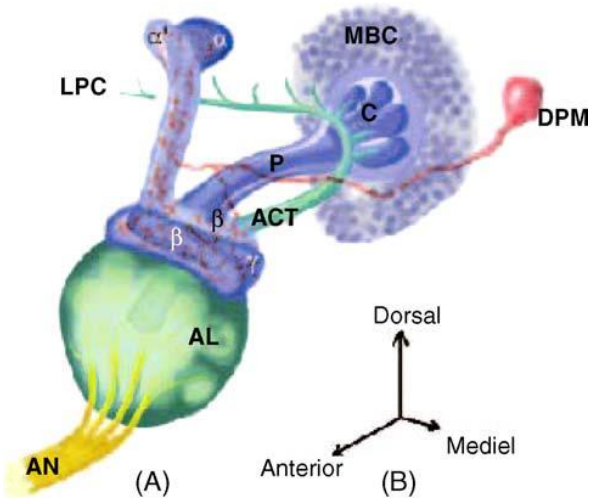

Figure 1. Image of the mushroom bodies of *Drosophila Melanogaster.* (McGuire, Deshazer, & Davis, 2005)

of discovery and the structural nuances of the mushroom body of the *Drosophila Melanogaster* brain provide a basis for exploring the vast scientific studies that have utilized it as a model. Beginning with experiments in learning and memory, the fruit fly has become a staple in many neuroscience labs and have even been used to explore the behavioral output of decision making.

Decisions of a Fruit Fly

So, as you, and the countless number of people that I have described this research to have wondered, what kind of decision does a fruit fly make? Unlike some of the lighthearted decisions we humans make in our day-to-day lives, the decisions a fruit fly makes can be crucial to their short one to two month lives. One of the most important decisions that *Drosophila Melanogaster* makes is the selection of where to lay their eggs. This decision, also known as oviposition, is crucial to the fecundity of the female and is an investment in her genetic future. When making this decision, numerous factors come into play including habitat choice, egg-load, and the availability of suitable oviposition sites (Betti, Soto, & Hasson, 2014). Evolutionarily, the behavioral output of oviposition site preference (OSP) has led to substantial species differentiation and scientists have proposed three hypotheses to describe the adaptive value of OSP. The first of these hypotheses entitled the ‘preference-performance’ hypothesis highlights the fact that females tend to choose to lay eggs in areas in which their offspring will have the highest performance once hatched (Betti et al., 2014). This hypothesis would lend itself to a positive correlation between offspring performance or survival and a more ideal OSP. The second hypothesis, entitled the ‘optimal foraging’ hypothesis states that females chose a site that actually increases their own survivability instead of that of their larvae. And finally, the third hypothesis, entitled the ‘free enemy space’ hypothesis describes how females choose a site that has the least number of predators that their larvae could possibly encounter (Betti et al., 2014).

Deciphering which hypothesis prevails can vary amongst species of this closely related subgroup of *melanogaster* that originated in sub-Saharan central Africa over around 5.1 million years ago (Betti et al., 2014). Though differing hypotheses exist as to its evolutionary origin, the behavioral choice of where to lay eggs is an energetically costly decision and the ability to compare various environments is obviously an adaptive benefit to the female fruit fly. Taking advantage of this important decision, neuroscientists can utilize techniques such as oviposition preference assays to assess oviposition site preference and eventually use it to explore how different genetic manipulations can affect this preference.

Oviposition preference assays take advantage of the female flies' desire to select an optimal oviposition site to explore which substrates are preferred. Once allowed enough time to make a decision and oviposit onto a selected substrate, eggs are typically counted on each substance to determine which one a fly prefers. This data is collected from a multitude of individual fly OSP's and compiled to portray a general preference. Researchers can then use this technique to explore a wide array of substances and compare the OSP of these substrates. For example, a research lab in 2008 explored the OSP between a sweet, sucrose-containing substrate and a bitter, lobeline-containing substrate and found that the females consistently preferred to lay eggs on the bitter surface (Yang et al., 2008). This preference, they go on to mention, was not due to a preference for lobeline (because flies consistently chose a plain substrate over lobeline) but rather, an avoidance of sucrose (Yang et al., 2008). Investigating this avoidance even further, these researchers found that sucrose was selected against no matter if the alternative choice was plain, lobeline, or sodium chloride (Yang et al., 2008). Establishing baseline preferences allowed these researchers to explore variations in these substrate assays including different concentrations of sucrose and even distance between each assay chamber (Yang et al., 2008).

With wildtype fly preference established, other labs could explore the effects of various chemicals and other substrates on oviposition site preference.

Explorations into Oviposition Preference

In an attempt to explore the effects of chemicals on *Drosophila Melanogaster* oviposition, one lab in 2016 decided to investigate the popular insecticide, Azadirachtin (Bezzar-Bendjazia et al., 2016). In order to test Azadirachtin's effect, researchers placed mated females into an assay containing an untreated medium and a medium treated with the aforementioned chemical. They found that flies demonstrate a significant preference for the untreated medium both in the case of the Azadirachtin medium containing 0.1μg and in the one containing 0.25μg (Bezzar-Bendjazia et al., 2016). Researchers also tested the effect of having no choice and found that females when presented with only the Azadirachtin medium showed a 30-40% decrease in eggs laid as compared to the untreated medium (Bezzar-Bendjazia et al., 2016). This research provides crucial evidence to support the evolutionary adaptation of vital decision-making neurons in the brain of the fruit fly that not only allow it to choose a proper place to lay its eggs, but to avoid a toxic one. Making critical decisions, as seen here, increase fecundity for *Drosophila Melanogaster* and allow for the neurons driving these behaviors to be favored by natural selection. Exploring yet another substrate, neuroscientists were able to determine the role that yeast plays in oviposition site preference. Known to be a nutritional support for larval growth and development, yeast could play an interesting role in triggering attraction and subsequent oviposition for *Drosophila Melanogaster.* Investigating this phenomenon, Becher et al. found that females are significantly more likely to oviposit onto grapes that were inoculated with yeast than on yeast-free grapes (Becher et al., 2012). This research shows that yeast scent is critical to attracting female flies and can alter the decision they make about where to oviposit.

Researching how different substrates like yeast can affect oviposition site preference is important for understanding the decision-making process and how differences can alter this choice.

Looking beyond the effects that differing chemicals and substrates can have on oviposition site preference, genetics can also play a major role in the decision-making process. Exploring the variation in phenylthiocarbamide (PTC) avoidance based on differences in genetics, researchers tested seven isogenic strains of *Drosophila Melanogaster* and found that though all strains avoided ovipositing onto PTC, the degree to which each strain avoided PTC had significant additive and nonadditive genetic variation (Possidente, Mustafa, and Collins. 1999). PTC, a synthetic thiourea compound that is similar to naturally occurring compounds found in cruciferous plants, has been shown to be toxic to fruit flies, so naturally, they avoid ovipositing onto a substance containing this compound. The curious part is the fact that each of the seven isogenic strains of *D. Melanogaster* avoided PTC to a different degree suggesting that these differences can be accounted for due to allelic variation (Possidente, Mustafa, and Collins, 1999). This finding not only demonstrates the difference genetics can play in fruit fly oviposition, but mapping these genes in fruit flies may directly correlate to genetic variation observed in humans for PTC preference and taste perception (Possidente, Mustafa, and Collins, 1999).

A more recent study from 2011 sampled over 5,000 flies from 295 wildtype fruit fly genotypes on their preference to lay eggs on either a nutritious substance with yeast or a nutritious substance without yeast in hopes of finding the difference genetics can play on this crucial decision (Miller et al., 2011). In their findings, these researchers detail how the average amount of eggs laid on each medium varied significantly between inbred lines, though most preferred to lay on the substrate without yeast and even more surprisingly, 15 genotype females

never laid a single egg on either substrate (Miller et al., 2011). This detailed study takes a major step toward understanding the natural, genetic variation that can occur in oviposition preference in *D. Melanogaster* and demonstrates how this variation can affect the decision-making behavioral output. Taking a step beyond merely examining the effect that genetics can play on decision making, researchers have begun linking behavioral outputs of *D. Melanogaster* to their brain neurons. One such study from 2015, examined the effect that *dsx*-expressing neurons exhibited on the behavior of oviposition (Kimura et al., 2015). *Dsx*-expressing neurons are known to play a critical role in 'female-specific functions' so by forcing their activation using dTrpA1, a warmth-sensitive channel, the researchers were able to study how they can affect behavior (Kimura et al., 2015). In this experiment, they found that activating *dsx*-expressing neurons induced egg laying in 80% of mated females and even in 50% of virgin females (Kimura et al., 2015). In their experiment, these researchers even found that without stimulation of the *dsx*-expressing neurons, no egg ejection was observed in females, most likely partially due to no suitable substrate being provided (Kimura et al., 2015). By highlighting one of the many roles that neurons play in controlling a fruit fly's behavior, these neuroscientists contributed to the field of decision-making science that may one day lead to understanding human behaviors. Though the neural circuitry of the fly brain is complex, and that of the human brain even more intricate, studies like the ones discussed here attempt to piece together an understanding of how specific sets of neurons can control female oviposition decisions.

Role of Dopamine in Oviposition Preference

Now that I have touched on the role that neurons in general can play in mediating oviposition preference, I wanted to delve into one specific type of neuron that has been found through extensive research to greatly impact the behaviors of *Drosophila Melanogaster* and even

mammals. Through its extensive arborizations innervating the brain, dopaminergic neurons have been implicated in their contribution to modulating fruit fly behaviors. One study examining how these dopaminergic clusters of cells can effect oviposition preference onto a substrate containing ethanol found that by blocking transmission of *TH-GAL4* and *Ddc-GAL4* (both sequences found in dopaminergic neurons), preference for oviposition changed as compared to wildtype flies (Azanchi, Kaun, & Heberlein, 2013). Interestingly, this study showed that blocking transmission of *TH-GAL4* which is found in only dopaminergic neurons increased preference for ethanol substrate by 5% whereas blocking transmission of *Ddc-GAL4* which is found in both serotonin and dopamine neurons actually decreased preference (See figure 2) (Azanchi, Kaun, & Heberlein, 2013). These stark differences in ethanol attraction and aversion demonstrate that there may be competing dopamine drivers modulating this contrasting behavior and that even a behavior as seemingly simple as oviposition can be very complex. Similarly, another study investigating the role of dopamine cluster neurons on sucrose preference found that by activating the *TH-GAL4* driver found in dopamine neurons, females preferred to oviposit onto substrates containing sucrose compared to plain substrates which is the opposite of what

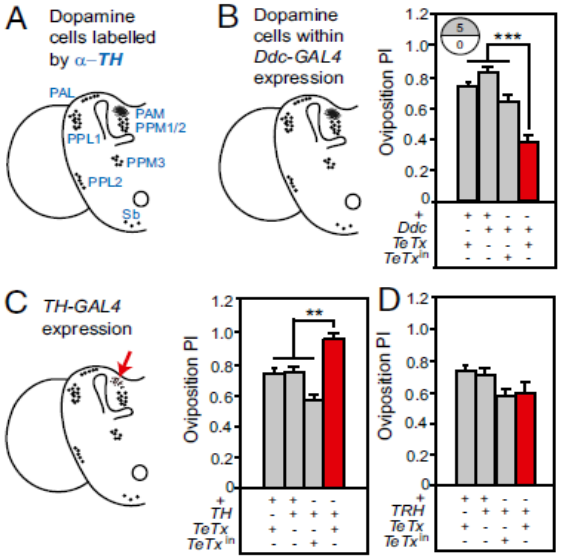

Fig. 2. Activity of dopamine and not serotonin neurons affects oviposition preference. (*A*) Dopaminergic neuron cell body positions in one hemisphere of the adult central brain are marked based on anti-TH immunohistochemistry (27, 28, 31, 32). PAM cell number is underrepresented in the schematic (33). (*B*) Cells colabeled with anti-TH antibody and *Ddc-GAL4* in the central brain (33). Disrupting neurotransmission in *Ddc* cells decreased oviposition preference [n = 17–21 per strain; ANOVA: $F_{(3,73)} = 47.51$, $P < 0.0001$]. (*C*) Schematic of the *TH-GAL4* expression pattern in the central brain (33). See Fig. S5 for more details. Arrow highlights that most PAM neurons do not express *TH-GAL4*. Disrupting neurotransmission in *TH* cells increased oviposition preference [n = 9–16 per strain; ANOVA: $F_{(3,51)} = 9.62$, $P < 0.0001$]. (*D*) Disruption of transmission in serotonergic neurons with *TRH-GAL4* did not disrupt oviposition preference [n = 19–21 per strain; ANOVA: $F_{(2,76)} = 4.23$, $P = 0.08$; all Tukey's comparisons to *TRH/TeTx*: $P > 0.05$]. Bars on graphs represent means ± SEM. $*P < 0.05$; $**P < 0.001$; $***P < 0.0001$. Clusters of dopaminergic neurons are named based on their location in the brain: PAM, protocerebral anterior median; PPL, protocerebral posterior lateral; PPM, protocerebral posterior median; PAL, protocerebral anterior lateral; Sb, subesophageal ganglion.

wildtype fruit flies prefer (Yang, He, & Stern, 2015). The design of their experimental assay ensures that no eggs are laid in an ambiguous 'middle-ground' so as to ensure a clear decision has to be made (See figure 3). Yang, He, and Stern hypothesized that this was due to an increase in value being given by the females to the sucrose substance when these dopamine neurons were activated. Because the *TH-GAL4* driver mainly labels dopamine neurons but can also label other types of neurons, these researchers went a step further to solidify that it was, in fact, dopamine neurons responsible for this finding. By activating the *TH-GAL4* driver and reducing expression in four known dopamine receptors, they were able to reduce preference for sucrose in three of the four dopamine receptors (Yang, He, & Stern, 2015). This result confirms their hypothesis that dopamine neurons play a major role in the altered preference of certain substrates and in this case, the preference for a sucrose containing substrate.

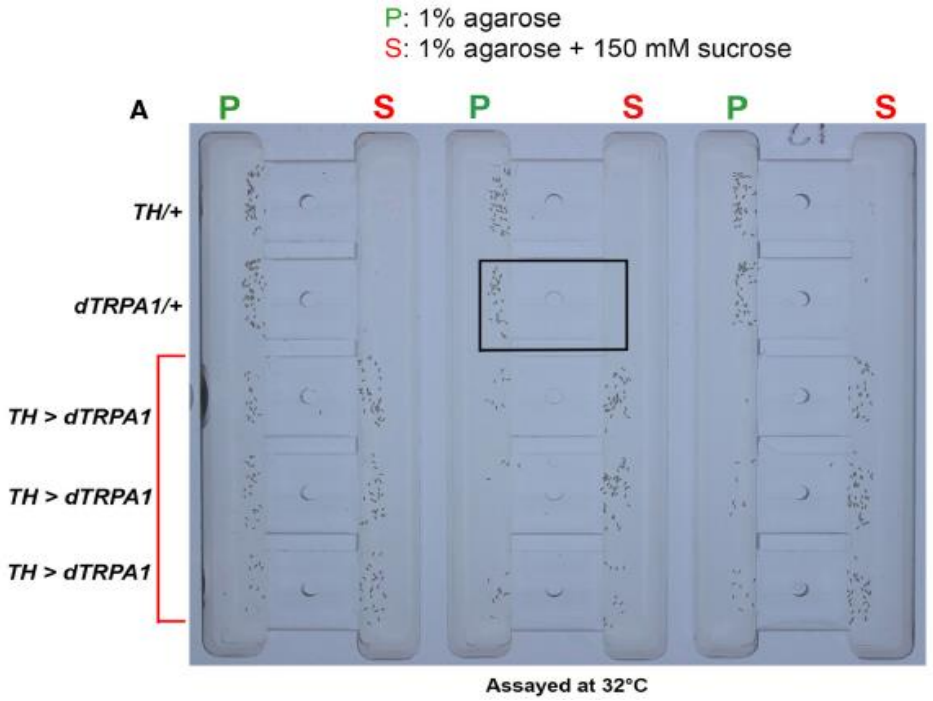

Figure 3. Activating the TH-GAL4-expressing neurons triggered a preference for laying eggs on the sucrose substrate in our sucrose (S) versus plain (P) chambers. Picture showing that when assayed at 32°C, control animals (top two rows) preferred to lay eggs on the plain substrates whereas animals with dTRPA1 expressed in their TH neurons (bottom three rows) preferred to lay eggs on the sucrose substrate. The boxed area in the middle denotes the area of a single egg-laying chamber.

Now that I have described the importance of studying decision making, why *Drosophila Melanogaster* is utilized as a scientific research model, what kind of decision a fruit fly makes, and how altering genetics can impact this ovipositional decision, I wanted to introduce the research I conducted in this field. Attempting to fill the gap in understanding how dopamine output neurons in the fruit fly mushroom body can impact decision making, my lab partner and I conducted a few experiments in the summer of 2019. One of the questions we were hoping to investigate was how do these dopamine neural circuits mediate influence ovipositional preference specifically of yeast and sucrose substrates? By investigating wildtype preference for these substrates, we were hoping to find a difference in the genetically altered fruit flies' preference. Another research question that we were looking into was which neuronal subsets were responsible for the change, if any, in oviposition preference? The answers to these questions are what my lab partner and I were hoping to find in the data we collected. Before beginning our research, we hypothesized that by silencing dopaminergic mushroom body output neurons, fruit flies will show a decreased preference for the plain substrate as compared to wildtype flies in a two-choice preference assay between the plain vs. sucrose-yeast substrates. In order to go about exploring these research questions and testing our hypothesis, the following methods were conducted.

Materials and Methods

Expanding fly stocks

Drosophila Melanogaster stocks were raised in a temperature-controlled setting of 20° C. We utilized the standard molasses and yeast food combination to maintain and raise the flies in typical plastic vials with cotton plugs. Adult flies were periodically transferred to new food every

5 to 7 days to ensure the health of the specimens. Both Wild-type and Mushroom Body (MB) output neuron knockout flies were maintained in this way. Within these stocks, virgin females were mated with males for 48 hours.

Wild Type preference assays

We conducted a two-choice oviposition preference assay developed previously in Dr. Sitaraman's laboratory. This assay consisted of a 20-chamber assay arena with 2 choices per chamber. In order to establish the standard wildtype oviposition substrate preference, we tested the following combinations of substrates: sucrose-yeast (SY) vs. plain (P); sucrose (S) vs. sucrose-yeast (SY); yeast (Y) vs. sucrose-yeast (SY); and plain (P) vs. yeast (Y). Once mated with males for 48 hours, female flies were collected and placed into the oviposition preference assay arena. Two mated females containing eggs were placed in each chamber and allowed to make a choice between two substrates for 6 hours at an assay temperature of 20° C. Preference was then measured using the oviposition preference index according to Flaven-Pouchon et al. (2014) by counting the number of eggs laid on each side of the chamber as follows:
Preference index: *(No. of eggs on substrate #1- No. of eggs on substrate #2) / Total eggs*

Dopamine Circuit experiments

Once wildtype preference was established, we decided to further pursue the substrate preference between sucrose-yeast (SY) vs. plain (P) because it was the clearest, most established preference. To investigate which dopamine circuits mediated this oviposition decision preference, we crossed KIR2.1 virgin female flies with select MB Output Neuron lines. KIR2.1 is a genetically encoded potassium channel that prevents neurons from firing action potentials by hyperpolarizing the cells. Crossing KIR2.1 genetically encoded flies with specific MB output neurons inhibited their function and allowed us to test which dopamine output neurons mediate

the oviposition preference. The same two-choice preference assay as previously described was conducted using the collected offspring of these crosses including the following output neuron circuits: MB433B, MB298B, MB434B, MB057B, MB549C, MB083B, MB027C, MB112C, MB082C, MB542B, MB077C, MB050B, MB543B, MB399B, and MB002B. Each of these strains varied on which dopamine output neurons were silenced. PBD flies were utilized as a control because these flies had 'junk' DNA inserted into their genome that did not code for any phenotypic results. This could act as a control because it simulated inserting DNA into the genome while not causing any phenotypic changes.

Results

Wild-type flies show oviposition preference for Yeast and avoidance of Sucrose

After testing wildtype flies using the two-choice preference assay, we analyzed the amount of eggs laid on each substrate using the Preference Index: *(No. of eggs on substrate #1- No. of eggs on substrate #2) / Total eggs*). A PI closer to 1 indicating a preference for the substrate listed first and a PI closer to -1 indicating a preference for the substrate listed second. There was a trend toward a preference for yeast and an avoidance of sucrose (Yeast vs. Sucrose-Yeast PI: 0.848; Yeast vs. Plain PI: 0.783; Plain vs Sucrose-Yeast PI: 0.916; Sucrose-Yeast vs. Sucrose PI: 0.028). Though this preference contrasts results found in other studies (Miller et al., 2011; Lihoreau et al., 2016) it can be assumed to be due to the influence that environmental cues, such as proximity to other food sources or amount of eggs to be oviposited, play on this important decision (Yang et al., 2008).

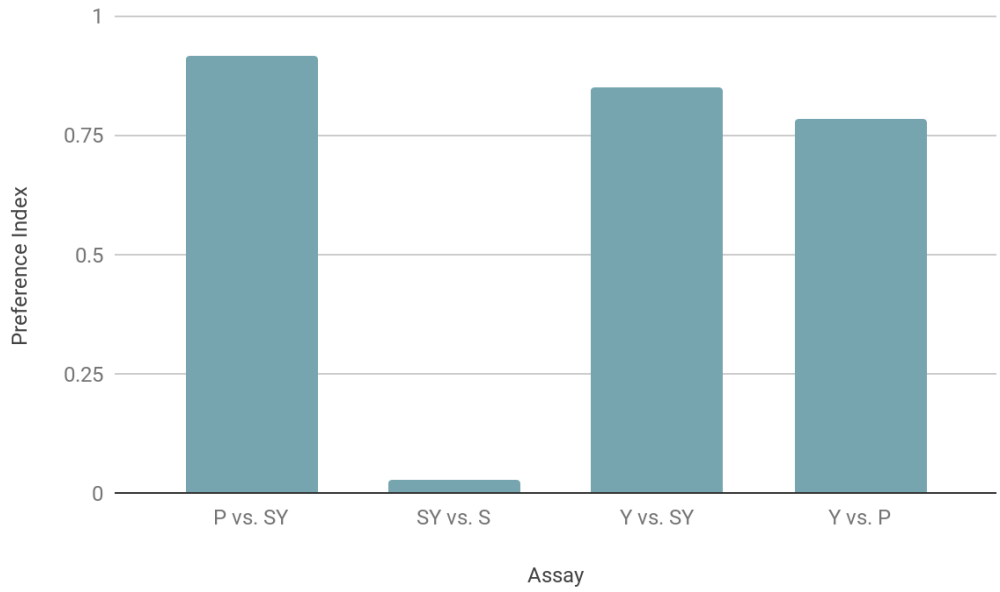

Figure 4. Wildtype Oviposition Preference Assay. Displays wildtype fly preference for yeast containing substrates and avoidance of sucrose containing substrates. Value closer to 1 indicates preference for substrate listed first below bar.

MB Output Neuron Preference

After establishing wildtype preference, we determined that Plain vs. Sucrose-Yeast had the strongest preference (PI: 0.916) and would be the best preference to explore in the Mushroom Body dopamine circuit knockout experiments. After crossing KIR2.1 virgin female flies with select MB Output Neuron lines (MB433B, MB298B, MB434B, MB057B, MB549C, MB083B, MB027C, MB112C, MB082C, MB542B, MB077C, MB050B, MB543B, MB399B, and MB002B) a preference remained for Plain vs. Sucrose-Yeast in these flies. However, a significant decrease in preference was observed in four of these lines (057B, 027C, 542B, 543B) indicating that they may play a more significant role in determining this preference for Plain over Sucrose-yeast.

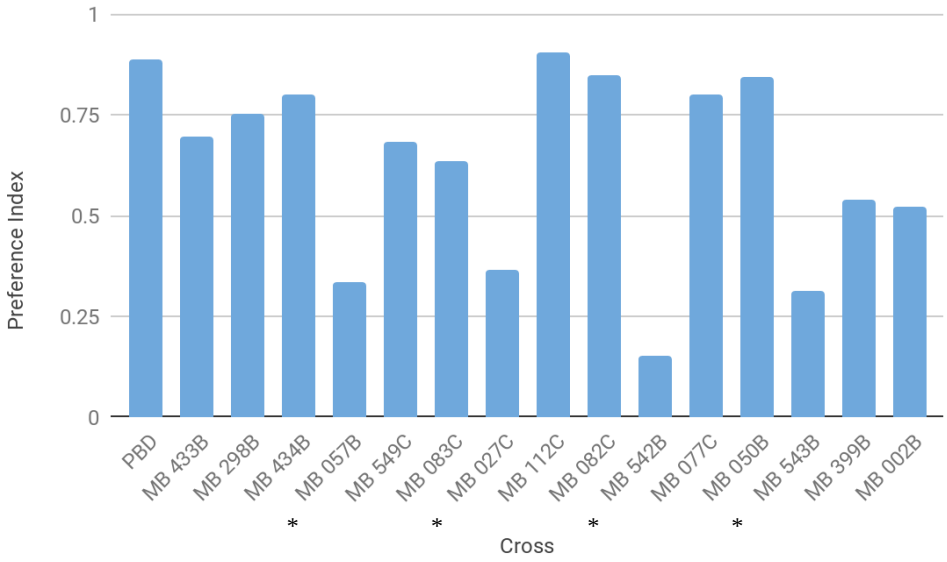

Figure 5. Mushroom Body Output Neuron Oviposition Preference Assay (Plain vs. Sucrose-Yeast). All lines still show preference for Plain, however 4 lines indicated with asterisks show a decreased preference.

Conclusion and Implications

In summary, we hypothesized that by silencing dopaminergic mushroom body output neurons, fruit flies will show a decreased preference for the plain substrate as compared to wildtype flies in a two-choice preference assay between the plain vs. sucrose-yeast substrates. To test this hypothesis, we first established the wildtype preference for yeast substrates and avoidance of sucrose substrates by testing four substrate combinations (Plain vs. Sucrose-Yeast, Sucrose-Yeast vs. Sucrose, Yeast vs. Sucrose-Yeast, and Yeast vs. Plain). We then found that by silencing certain dopaminergic mushroom body output neurons, mutated flies still prefer Plain substrate in a test of Plain vs. Sucrose-Yeast, however, in four subsets of neurons (057B, 027C, 542B, 543B), fruit flies showed a decreased preference for Plain, meaning they chose the

Sucrose-Yeast substrate more often than in wildtype flies, indicating that these subsets could play a role in mediating this ovipositional response.

Wildtype flies show preference for yeast and avoidance of sucrose

Our results indicate that when placed in a two-choice preference assay, wildtype flies tend to prefer yeast and avoid sucrose. Many sources tend to help explain the latter result. Though rejection of sucrose, a natural energy source, may seem evolutionarily counterproductive, as it could provide a food source for young fly larvae, it may also attract predators that could harm the fruit fly's offspring. Not only this, but as a fruit fly is depositing its eggs onto a surface, it remains vulnerable to attack itself, which may prove evolutionarily detrimental (Miller et al., 2011; Yang, He, & Stern, 2015). Avoiding the sugar containing substrate, therefore, may prove to be a more evolutionarily stable option for *Drosophila Melanogaster.* The preference for a yeast containing substrate, on the other hand, may be due to the female flies' decisions that it provides reward rather than risk. The protein and nutritional benefits of yeast confer evolutionary benefits onto the larvae of the fruit fly as these larvae tend to remain close to the site upon which they are laid and proximal nutritional sources are required (Chin et al., 2018). In the case of both sucrose and yeast containing substrates, olfaction plays a key role in how fruit fly females determine an optimal oviposition site. The olfactory set of neurons found in the fruit fly must take in external cues about various substrate chemicals and rank them in order to determine the optimal oviposition site (Chin et al., 2018). Our findings further emphasize the balancing act that occurs within the fruit fly olfactory system in order to find a nutrient-rich substrate that can support the survival of larvae but not attract nutrient-seeking predators.

Mushroom Body Output Neuron Preference

Our results for the Mushroom body output neuron silenced flies indicate a significant decrease in preference for the plain substrate in four of the 15 lines we tested in the two-choice preference assay of plain vs. sucrose-yeast substrates. As mentioned previously, wildtype flies tend to avoid substrates containing sucrose due to the predator-attraction and increased risk to their offspring, and so because of this, chose the plain substrate more in our assay. With specific strains of dopaminergic mushroom body output neurons silenced, we were hoping that these flies would have a complete reversal of preference and chose the sucrose-yeast containing substrate instead of the plain substrate. Though this result was not found, we did see a significant decrease in preference in four of the 15 tested lines (057B, 027C, 542B, 543B). Interestingly, both 542B and 543B strains of output neurons are expressed in the α′1 region of the mushroom body, indicating that this region, more specifically, may play a role in modulating oviposition site preference. These images depict the fruit fly mushroom body brain structure as seen in purple, with the individual subsets of dopaminergic output neurons highlighted in green (See Figure 6) (FlyLight Split-GAL4 Driver Collection., 2014). As you can see, both are in the same region of the mushroom body structure (the α′1 region).

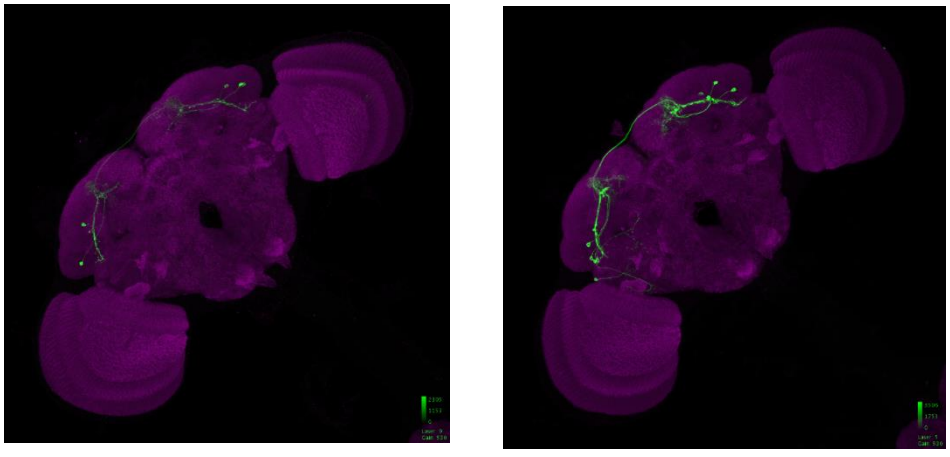

Figure 6. Mushroom Body Output Neuron 0542B (left) and 0543B (right)

Implications: Connection to mammals and humans

After discovering previous studies as well as conducting my own research on the effects of dopamine on fruit fly oviposition, I have found that this neurotransmitter can have great effects on fly behavior (Azanchi, Kaun, & Heberlein, 2013; Yang, He, & Stern, 2015). But how does this finding affect humans and the world we live in? Not only can dopamine neurons play a role in modulating oviposition preference for *Drosophila Melanogaster,* but effects of these dopamine neurons can be found in mammals as well. A study using Long Evans rats examined the effects of selective dopamine agonists (D1, D2, and D3) or corticotropin releasing factor (CRF) on the ability of the rats in an operant based effort procedure (Bryce & Floresco, 2019). The main findings of this study were that stimulating the D2 receptors shifted the rats' selection away from larger, more costly rewards in favor of smaller, easy to obtain rewards (Bryce & Floresco, 2019). This study shows that changing dopamine levels can impact the decision that these mammals make. In a similar study, this time examining the effects of combining a dopamine reuptake inhibitor with a noradrenaline reuptake inhibitor to test for redundancies in monoaminergic function, researchers found that combining the increase of both dopamine and noradrenaline caused rats to shift from advantageous decisions to disadvantageous decisions in a rat gambling task (See Figure 7) (Baarendse, Winstanley & Vanderschuren, 2012). In both of these

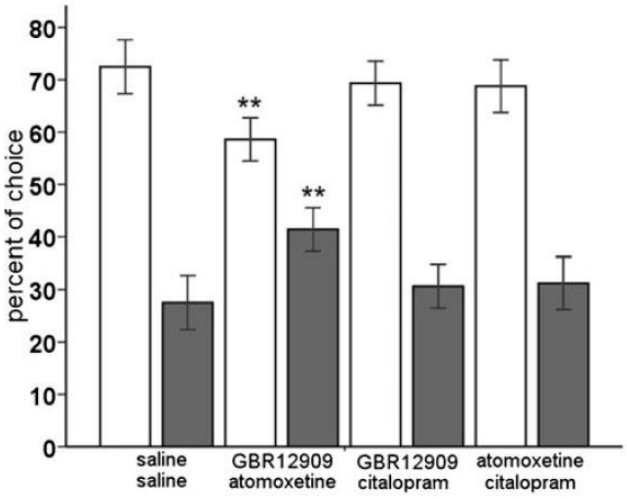

Fig 7 Effects of combined administration of the selective DA reuptake inhibitor GBR12909 (10 mg/kg), the selective NA reuptake inhibitor atomoxetine (3 mg/kg), and/or the selective 5-HT reuptake inhibitor citalopram (3 mg/kg) on choice behavior, i.e., advantageous options versus disadvantageous options in the rGT. In total, $n=20$ animals were included in the analysis. $^{**}p<0.01$ compared to vehicle treatment (paired samples t test). All data are expressed as mean±SEM

studies, dopamine was shown to play a key role in modulating mammal behavior. In humans, more specifically, dopamine dysregulation has been shown to factor into the cause of several mental disorders. Irregular levels of dopamine impact decision-making skills which, in turn, has proven to be a main problem for humans with drug addiction, schizophrenia, ADHD, Parkinson's Disease and pathological gambling (Baarendse, Winstanley & Vanderschuren, 2012). Understanding the role of dopamine in decision making processes using *Drosophila Melanogaster* as a model for study proves vital to the research behind the mechanism and eventual cure of these mental disorders.

Strengths and weaknesses

One strength of the research that I conducted is the reliability of the data that was collected for the wildtype preference assays. When conducting research, reliability refers to the overall consistency of the data due to replication of the experiment. In conducting the wild type two-choice preference assays, my lab partner and I combined our data with the data collected previously in Dr. Sitaraman's lab to produce, overall, more than 30 replications of each two-choice substrate preference assay (sucrose-yeast vs. plain; sucrose vs. sucrose-yeast; yeast vs. sucrose-yeast; and plain vs. yeast). Conducting a high number of assays of each substrate combination allowed us to take an average preference index of the combination to create more reliable data. Reliability of data is important because it shows that the data collected in consistent across tests and the meticulous replication of experimental environmental factors during each assay allowed our results to be reliable. Another strength of our research was the number of mushroom body output neuron subsets that we tested for their effect on oviposition preference. By testing 15 different subsets, we were able to examine a greater region of the mushroom body in order to properly assess the modulating effect that these dopaminergic neurons may play on

fruit fly oviposition. A third strength of our research was choosing to examine genetically altered fly preference for the Plain vs. Sucrose-Yeast substrates because this combination had the clearest preference outcome in the wildtype preference assays. Choosing to focus our research on this combination only, we were able to compare any results that we found against the clearest preference established for the wildtype flies. This provided a way to more clearly observe any potential differences in preference that the altered flies may exhibit. These three strengths allowed our research to collect important data that will one day aid in creating a more complete map of the fruit fly mushroom body output neurons and their modulating functions.

One weakness that may have been a limitation in our research is the distance between the two choices of substrates. Described in several research articles, the context in which oviposition occurs can play a major role in the substrate that is chosen. For example, larger distances between nutritional substrates in nature increase energy spent in sampling each of these and may cause the fruit fly to simply choose to oviposit on the first substrate found, regardless of its predator-attracting properties (Chin et al., 2018). Because the two-choice preference assay was built to be relatively small with only a few centimeters between each option, fruit flies may have been able to spend more time and energy exploring each one to make an optimal choice. This may be opposite to what occurs in nature. Examining this exact phenomenon, one study found that when female flies were placed into an assay with either two substrates on opposite ends of a petri plate or into an assay with the two substrates right next to each other in the center of the petri plate, distance explained 63-78% of all of the variance in four different genotypic lines of fruit fly oviposition site selection (Miller et al., 2011). With only a few centimeters between preference choices in our assay, this finding could prove to be a limitation in our wildtype preference findings due to the lack of distance between our two choices within the oviposition

assay (See Figure 8). Another weakness of our research could be oversimplification for the purposes of lab research. In order to examine the specifics of substrate preference in oviposition, we had to simplify each substrate choice to either one substrate or a simple concentration of two (sucrose-yeast). Once again, in nature, there may not be this simple of a choice to be made. As pointed out in the Miller et al. study, a finding that seems ground-breaking in the laboratory, experimental setting may still be very cryptic in nature due to a combination of multiple substrates, location of these substrates, competition for an ovipositional site and number of predators (2011). A final weakness of our research was the lack of time and funding to test genetically altered flies for their preference in the other substrate combinations including Sucrose-yeast vs. Sucrose, Yeast vs. Sucrose-Yeast, and Yeast vs. Plain. Testing these other substrate combinations could have given us a wider array of data that would have allowed us to examine the modulating effect that these neurons play on oviposition preference of other substrate combinations. Though these three weaknesses may provide alternative explanations for our results and may have limited the full potential of the data we could have collected, the results that we did find, nevertheless, still contribute to the formation of a more complete map of the mushroom body output neurons that modulate fruit fly oviposition preference.

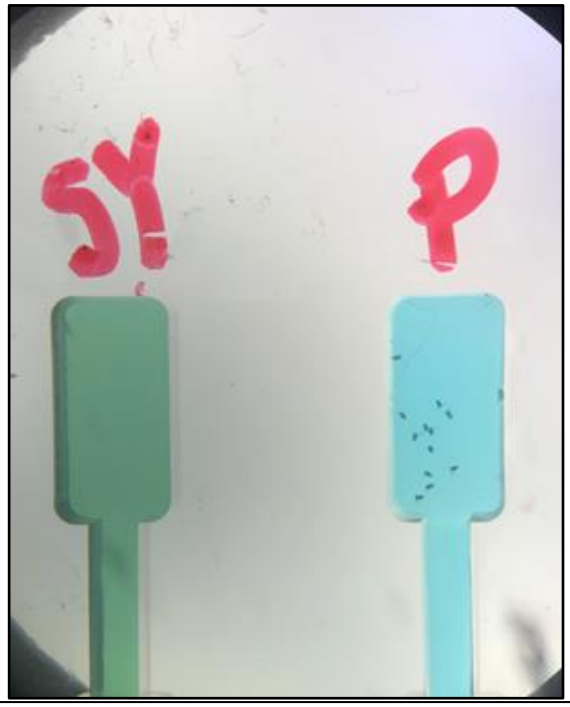

Figure 8. Image of the ovipositional assay examining preference between Sucrose-Yeast and Plain substrates in our research.

Future Directions

Due to the time constraints on our original research, a future study could further examine how the KIR2.1x MB Output Neuron crosses effect ovipositional choice of the other three substrate choices (sucrose-yeast vs. sucrose; sucrose-yeast vs. yeast; and yeast vs. plain). This future study could utilize our same methodology and compare the results found to the ones we found using wildtype flies. The data collected from this future study would not only further complete the research we began by adding information to how KIR2.1x MB Output Neuron crosses impact the substrate decision, but it would also expand the data of the neuroscience decision-making field. Understanding how these crosses impact additional substrate combinations would provide a more comprehensive look into the impact of dopaminergic mushroom body output neurons on ovipositional preference. I hypothesize that offspring of these KIR2.1x MB Output Neuron crosses would similarly avoid sucrose and chose substrates containing yeast but that, the same four types of MB output neurons that showed a decreased preference for plain (057B, 027C, 542B, 543B), would also show a decreased preference for the choices that wild type flies would normally choose. These four MB output neuron lines have already been implicated in playing some role in controlling the decision that fruit flies make in where to lay eggs, so I expect them to also play a role in these other three substrate preferences.

Final Thoughts and Conclusion

Upon examining our hypothesis that silencing dopaminergic mushroom body output neurons would cause these genetically altered fruit flies to show a decreased preference for the plain substrate as compared to wildtype flies, we found that four of the 15 genetic lines we examined did show this decreased preference. This alteration in fruit fly oviposition behavior due to a silencing of dopaminergic neurons provides support of their possible role in modulating

decision-making behaviors. Connecting these dopamine lines in fruit flies to the ones found in mammals, and hopefully one day to the ones found in humans, researchers remain optimistic that understanding the neuroscience behind everyday decisions that we make may not be too far off. Though there is still plenty of work to be done in connecting these dopaminergic mushroom body neurons to the behaviors they mediate, I, myself, and others in the field continue to have hope that one day, we will fully understand the role that neurons play in human decision making processes.

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