EFFECTS OF SENSORY STIMULI ON MEMORY IN ALZHEIMER'S *DROSOPHILA* MODELS

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1 Acknowledgements

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2 Abstract

Over 5.7 million Americans are affected by Alzheimer's disease (AD). The symptoms of AD often cause anger in patients and inhibit their interactions with others. Many patients rely on therapies to provide palliative care. Studies have shown that therapy with auditory stimulations improves cognitive function in patients dealing with AD. This project looks at whether other sensory stimulations, specifically optic and olfactory stimulations, elicit similar responses to the auditory stimulations. These stimulations were tested on flies. Each stimulation was tested with a learning assay in which the flies learned to avoid a positive stimulus. A t-test was run to analyze the data and compare the groups. The olfactory stimulations were found to improve memory ($p = 0.000396$). The optic stimulations were also found to improve memory ($p = 0.001052$). Further studies are being conducted in flies with genes associated with AD. This approach should be integrated into therapies for AD patients, to help improve the memory of the patients. The results are currently being integrated into a reminder system for the elderly and dementia patients, with an optic stimulus being used as the reminder.

3 Literature Review

3.1 Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disease that influences behavior, cognizance, and ability to remember information. Approximately 5.7 million Americans are living with Alzheimer's disease. While the majority of people affected by Alzheimer's are 65 years or older, it also affects approximately 200,000 Americans under the age of 65. It has become the leading cause of dementia in adults, increasing mortality in the elderly. The disease causes degeneration of brain tissue and nerve cells, killing cells used to retrieve and process information. The cause for this degeneration is not currently known, however, scientists have identified numerous possible causes (What is Alzheimer's, n.d.).

3.2 Possible Causes of Alzheimer's Disease

A putative cause is the growth of amyloid plaques in the brain. Amyloid plaques are aggregates formed in between nerve cells from misfolded beta-amyloid protein fragments (Figure 1). The development of these aggregates is generally attributed to the fragmentation of the amyloid precursor protein (APP) by beta secretase and gamma secretase. While Amyloid plaques normally develop with age, excess growth has been observed in Alzheimer's patients. These plaques are believed to disrupt communication between the nerve cells.

Another potential cause of Alzheimer's disease is the formation of neurofibrillary tangles in the brain (Figure 1). Neurofibrillary tangles are twisted fibers of the tau protein, a protein used to sustain microtubules in neurons. Unlike amyloid plaques, which occur outside

the cells, neurofibrillary tangles occur inside the cells. These tangles naturally develop in healthy brains, however, similar to amyloid plaques, they display excessive growth in Alzheimer's patients. Neurofibrillary tangles occur when the molecules of the tau protein disaggregate into filaments which tangle. As a result, the microtubules in neurons lose the interconnectivity vital to transport essential materials for the neuron's survival. Eventually, the connection between the neurons is completely disconnected, as the neurons keep dying (Alzheimer's Brain Tangles, n.d.).

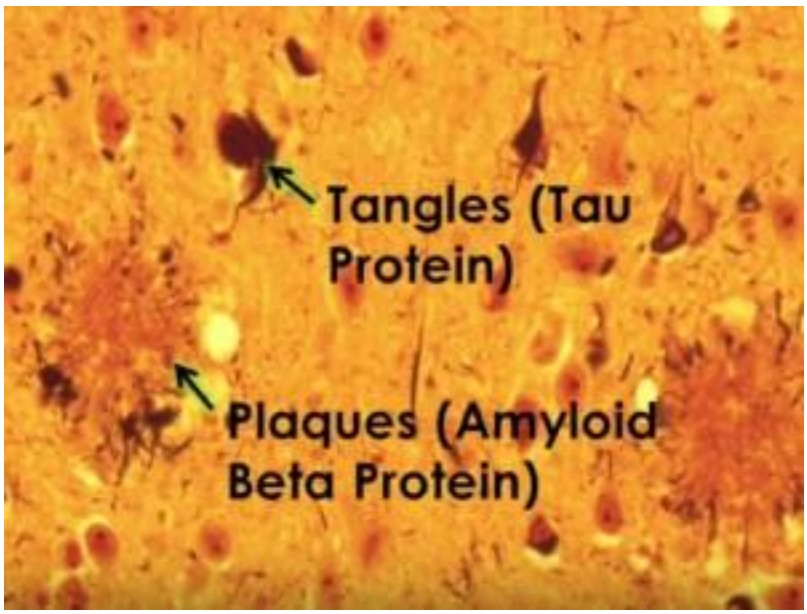


Figure 1: Beta-Amyloid Plaques and Tangles ("Modulating the Genetic Factors…", 2017)

3.3 Symptoms of Alzheimer's Disease

Alzheimer's patients experience a range of symptoms. The first symptom most people experience is memory loss, due to the severe loss of connections between neurons. Patients often have difficulty remembering recent events, while also having trouble with learning new information and new cognitive abilities. Due to the fact that they often have trouble with

memory and can get confused easily, Alzheimer's patients often get frustrated and angry. This emotional response causes problems during their interactions with other people. Alzheimer's disease can also impair a person's ability to communicate, as patients in later stages often have trouble speaking. The symptoms of the disease become more severe as it progresses, eventually leading to the patient's premature death (Memory Loss, 2009).

3.4 Alzheimer's Disease Treatments

While no existing treatments can completely cure Alzheimer's disease, there are drugs which help to slow down the pace at which the disease progresses. These drugs help to slow and stop the process of amyloid beta plaque generation, significantly prolonging the patient's lifespan. Two types of drugs, cholinesterase inhibitors and memantine, have been approved by the FDA to moderate Alzheimer's disease symptoms. These medications treat memory loss and cognitive abilities by preventing the breakdown of certain chemicals in the brain, which help with communication among neurons (Medications for memory, n.d.).

Many companies are working to implement various therapies for Alzheimer's treatments. Elan Corporation completed a study which tested the effectiveness of an immunotherapy treatment. The study was conducted on amyloid mice which were injected with beta-amyloid fibrils. The mice displayed reduced plaque pathology, indicating that small quantities of beta-amyloid fibrils can help to clear beta-amyloid deposits. Another treatment being investigated is one based on epidemiology. Some epidemiological studies show that non-steroidal anti-inflammatory drugs (NSAID) have an effect on Alzheimer's disease, as it is

believed that Alzheimer's is partly caused by chronic brain inflammation. Some NSAIDs have been shown to reduce production of beta-amyloid plaques, a potential cause of Alzheimer's disease (Citron, 2002).

3.5 Therapy Treatment

Due to the fact that no cures for Alzheimer's disease exist, many patients turn to therapy. Music therapy relies on auditory stimulation in order to help alleviate some of the symptoms of Alzheimer's disease. Alzheimer's patients who received the therapy biweekly have shown increased orientation, language, and memory. The therapy also improved cognitive function in the patients as demonstrated by increased mini-mental state examination scores (Gómez Gallego & Gómez García, 2017).

3.6 Drosophila melanogaster

Typical model organisms used to study neurological diseases are *C. elegans* (roundworm), *Danio rerio* (zebra fish), and *Drosophila melanogaster* (fruit flies). *C. elegans* are relatively expensive to do research on, as a microscope and other equipment are required for close observation, while *Danio rerio* can only be used for 6-day cycles as they become vertebrates and must be disposed of. In comparison, *Drosophila* are cheap to maintain, easy to handle given their small size, produce many offspring, and are available in many modified genetic strains. Even though *Drosophila* are small, it is still easy to separate male and female flies with the naked eye as the male has a dark spot on the tip of its abdomen (Figure 2). More importantly, *Drosophila* have brains containing 200,000 neurons which are similar to those of

humans. *Drosophila* receive almost all of their sensory input through visual and olfactory stimulations and rely on these sensory inputs for learning and locomotive abilities. Because this model is powerful, easily accessible, and cost effective, there have been many previous studies and experiments done on *Drosophila* before, which provide a useful catalogue of past data and assays. For these reasons, *Drosophila* are often used as model organisms for Alzheimer's research (di Carlo, 2012).

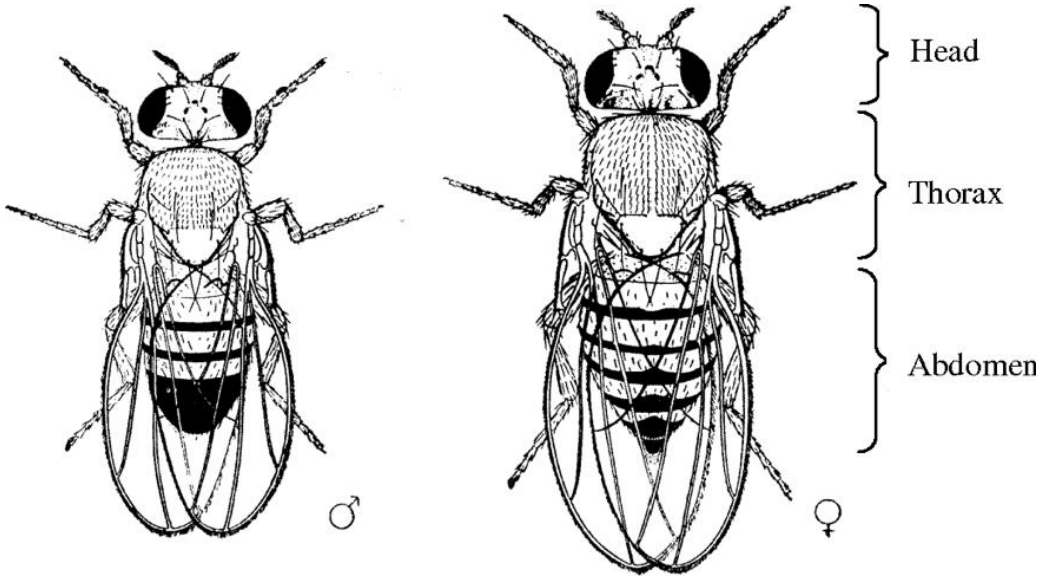


Figure 2: Drosophila melanogaster (Yee, 1970)

3.7 *Drosophila* Life Cycle

Adult *Drosophila* will mate, and the females are able to lay up to 100 embryos per day, resulting in many embryos after a few days of mating. The embryos mature through several stages of larvae. After seven days, these larvae will begin roaming, with the adult flies emerging within eleven to twelve days (Figure 3). The female flies will become sexually mature within 8-

10 hours after emerging. This cycle provides the researcher with an abundance of flies to use when running experiments (An introduction to fruit flies, 2015).

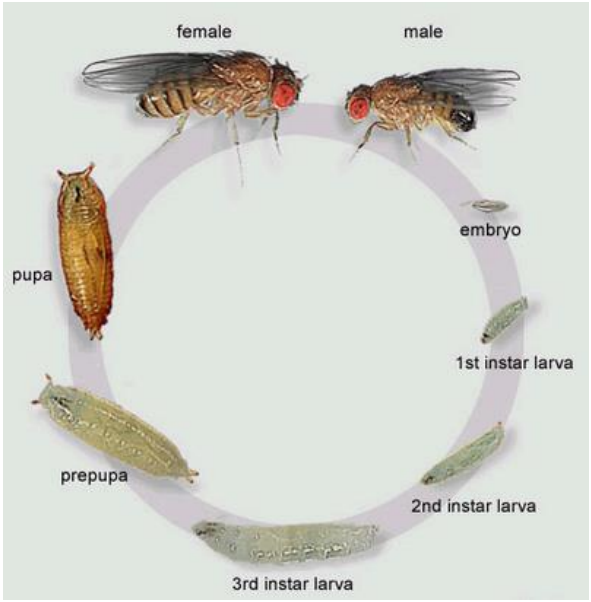


Figure 3: Drosophila melanogaster life-cycle (Drosophila melanogaster is an enemy…, n.d.)

3.8 *Drosophila* as an Alzheimer's Model Organism

As mentioned above, *Drosophila melanogaster* are often used as model organisms to study human diseases. *Drosophila* contain many genes similar to those in humans which are associated with human neurodegenerative diseases. Research has shown that 75% of the genes related to most human diseases can be found in *Drosophila*, making them optimal for learning about human diseases (Moloney, 2010). The Gal4 / UAS gene expression system is typically used to help breed fruit flies with the genes of interest. In this system, one line of flies carries the gene of interest with the UAS enhancer, while another line of flies is kept with the Gal4 driver and a tissue specific promoter (Figure 4). The tissue specific promoter explicitly targets regions where the gene needs to be expressed. Flies from these two lines are crossed, leading

to the Gal4 protein binding with the UAS and activating the gene related to the tissue specific promoter. This system can be used to overexpress genes of interest.

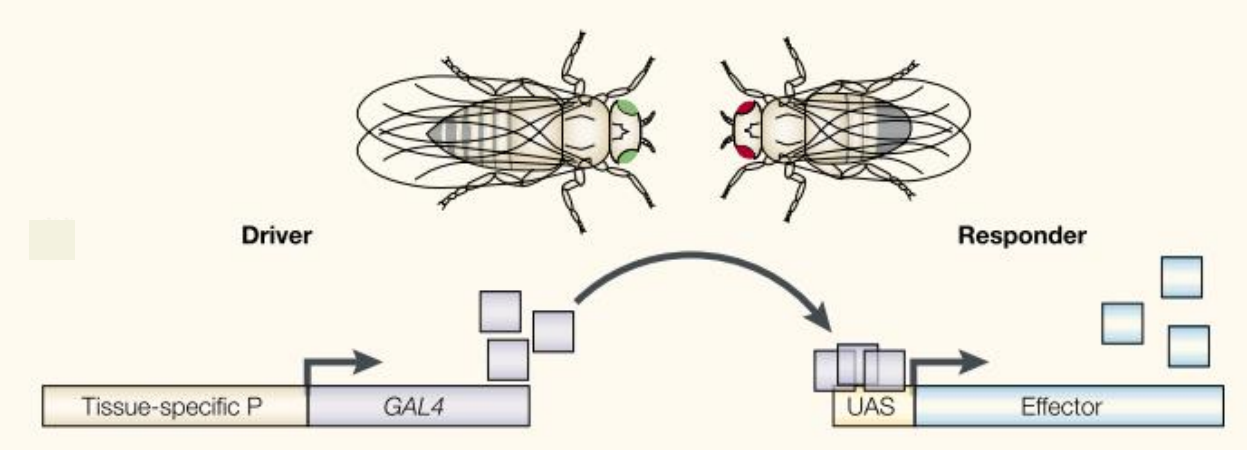


Figure 4: Gal4/UAS system (Dpp-Gal4…, 2013)

There are many documented assays to use when working with *Drosophila* in order to test a variety of behaviors. Two such behaviors which Alzheimer's disease researchers might want to study are locomotion and memory. Locomotion can easily be tested using a Rapid Iterative Negative Geotaxis (RING) assay, whereas memory can be tested using an aversive phototaxis suppression assay. The RING assay provides information on locomotive ability by testing how many flies can climb to a certain height in three seconds. The aversive phototaxis suppression assay teaches flies to become negatively phototactic, meaning they avoid light, based on an aversive stimulus. There are a variety of aversive stimuli that can be used in the aversive phototaxis suppression assay, such as shaking or electric shock (Ali, Escala, Ruan, & Zhai, 2011). These aversive stimuli are used to teach the *Drosophila* to avoid certain environments or situations. The short time between each *Drosophila* generation allows for many flies to be tested at once and for more flies to continue being bred from one stock.

4 Plan

Researchable Question: How do olfactory and visual sensory stimulations affect memory recall in *Drosophila* models of Alzheimer's disease?

Hypothesis: If *Drosophila* models of Alzheimer's disease are exposed to olfactory and visual sensory stimulations, then they will exhibit increased memory recall.

5 Methodology

5.1 Materials

Material	Source
Drosophila Medium	University of Massachusetts Medical School
Incubator	Dr. Duffy's Lab
Sucrose Solution 0.5M	Carolina Biological
Ethyl Alcohol 95%	Innovating Science
T-maze	3D Printed at Mass Academy
Drosophila melanogaster Bloomington stock #64384, expresses APP under UAS control	Bloomington Drosophila Stock Center
Drosophila melanogaster Gal4 strain	Bloomington Drosophila Stock Center
Paint Brush	Dr. Duffy's Lab
Drosophila Vials and Bottles	Mass Academy and Dr. Duffy's Lab
Plexiglass Sheet	Previously owned
Blue LED Lights	SumDirect
Filter Paper	Dr. Duffy's Lab
Microscope	Dr. Duffy's Lab
CO_2	Dr. Duffy's Lab
CO_2 Anesthetizing Pad	Dr. Duffy's Lab
Yeast	Dr. Duffy's Lab

Table 1: List of all the materials used and where they were obtained from

5.3 Drosophila Care

The Drosophila were kept in an incubator with an automatic 12-hour light/dark cycle. The incubator was set at 26.5 °C in order to speed up population growth. Flies were kept in bottles with pre-made medium from University of Massachusetts Medical School, Worcester.

In order to quickly increase the population size, adult flies were transferred to new bottles with fresh food every two to three days. The larvae were left in the old bottles allowing for easy collection of virgins. To transfer the adult flies, the old bottle was tapped until the flies fell down to the bottom. Then the foam stopper on the top of the bottle was removed and a new bottle was placed on top of the old bottle. The flies were then tapped into the new bottle and a foam stopper was quickly placed on the new bottle. The new bottle was labeled with the appropriate strain name and date in order to prevent confusion. Then a foam stopper was placed on the old bottle and both bottles were placed back in the incubator.

5.4 Sexing Drosophila

The *Drosophila* were sexed, and the virgins were collected to be used for expressing APP. The flies were first knocked out using the CO_2 anesthetizing pad. They were emptied into a funnel onto the CO_2 pad where they were knocked out. They were then spread out over the pad and placed under the microscope to be observed clearly. In order to distinguish the male *Drosophila* from the females, the tips of the abdomen were observed. The tips of the abdomen of male *Drosophila* are solid black. The female Drosophila also have more pointed tips, while the males are more rounded. Then, the female virgins were collected. Two factors were used to decide whether a female was a virgin. Virgins generally have lighter color abdomens than other flies. Another indicator used to identify virgins was a black mark that is found on the abdomens of all virgin *Drosophila*. One simple indicator that a fly is not a virgin is if it has an egg protruding from the tip of its abdomen. Using these criteria, the virgins were collected and brushed into

separate vials using a paint brush. This process was repeated every 8 hours until enough virgins were collected to perform a cross.

5.5 Crossing *Drosophila*

Once enough virgin *Drosophila* were collected from the UAS strain (refer to section 5.4), they were placed in a vial with male *Drosophila* from the Gal4 strain. The flies were left together in the vial until they mated, and the females laid embryos. In order to encourage mating, the cotton stopper at the top of the vial was pushed down causing the flies to interact more in a confined space. The flies were also kept at 26.5 °C in order to accelerate the population growth. Once the eggs were laid, the adult flies were flipped to another vile. This allowed the adults to continue mating while giving the eggs space to grow. This process was repeated until sufficient progeny for experimentation had developed.

5.6 Apparatus

A T-maze (Figure 5) was used to test the learning and memory of the flies. The T-maze had two chambers separated by a divider and was covered with a plexiglass sheet to prevent the flies from escaping. A piece of filter paper soaked in either the ethanol or sucrose solution was placed in once chamber, while the other chamber was left empty. Lights were placed in holes on either side of the T-maze. Flies were placed in the T-maze by connecting the *Drosophila* vial to the opening in the T-maze.

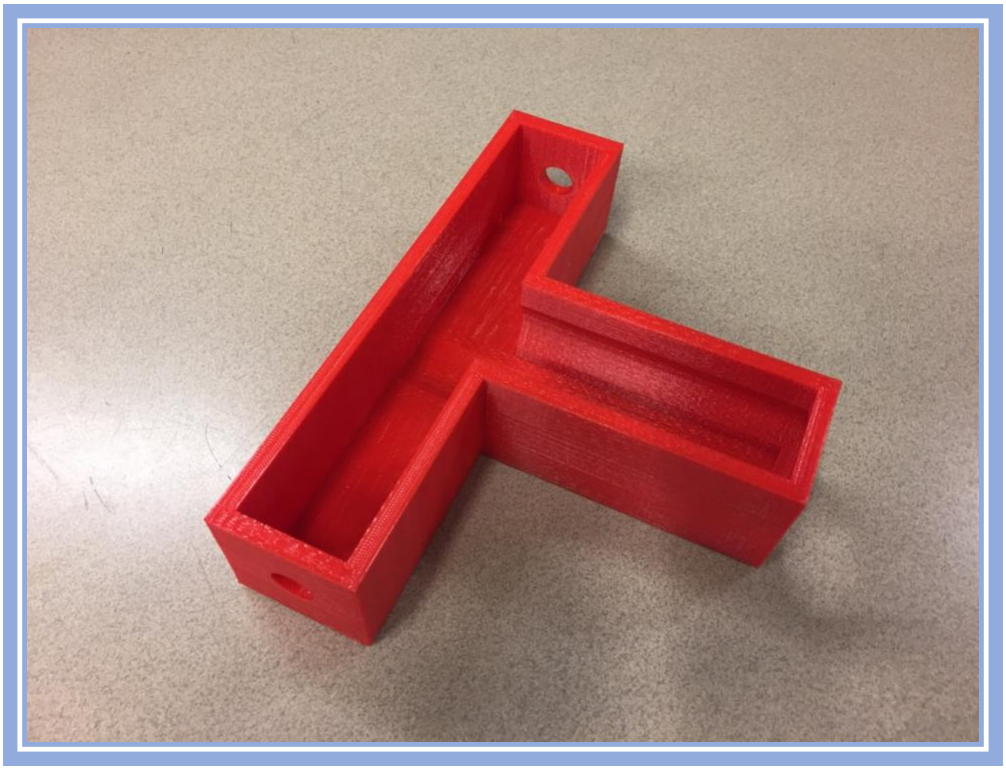


Figure 5: T-Maze Apparatus for Memory Assays

5.7 Aversive Phototaxis Suppression Assay

First, the T-maze was prepared. A light was placed in the hole at the end of one chamber along with a piece of filter paper soaked in ethanol. The other chamber was left empty and dark.

The flies were then split into six groups. The first was the control group, and the other five were the experimental groups. The control group was trained to become negatively phototactic by exposing the flies to the light source while the T-maze was being shaken for one minute. After ten minutes, the flies were reintroduced into the T-maze and given ten seconds to choose which chamber they would go into, the lighted one or the dark one. The number of flies that failed to walk into the lighted chamber was recorded. This number was divided by the

total number of flies to provide the passing rate. This same procedure was used for the experimental groups with some slight differences. For the experimental groups, the training consisted of the flies being exposed to the light source and the ethanol-soaked filter paper while the T-maze was being shaken for one minute. Then, while the flies were reintroduced to the T-maze, they were once again exposed to the ethanol-soaked filter paper. The number of flies that failed to walk into the lighted chamber was recorded. This number was divided by the total number of flies to provide the passing rate for each experimental group.

5.8 Optic Stimulation Aversive Stimulus Learning Assay

First, the T-maze was prepared. A blue light was placed in the hole at the ends of either chamber. A piece of filter paper soaked in sucrose solution was placed in one of the chambers.

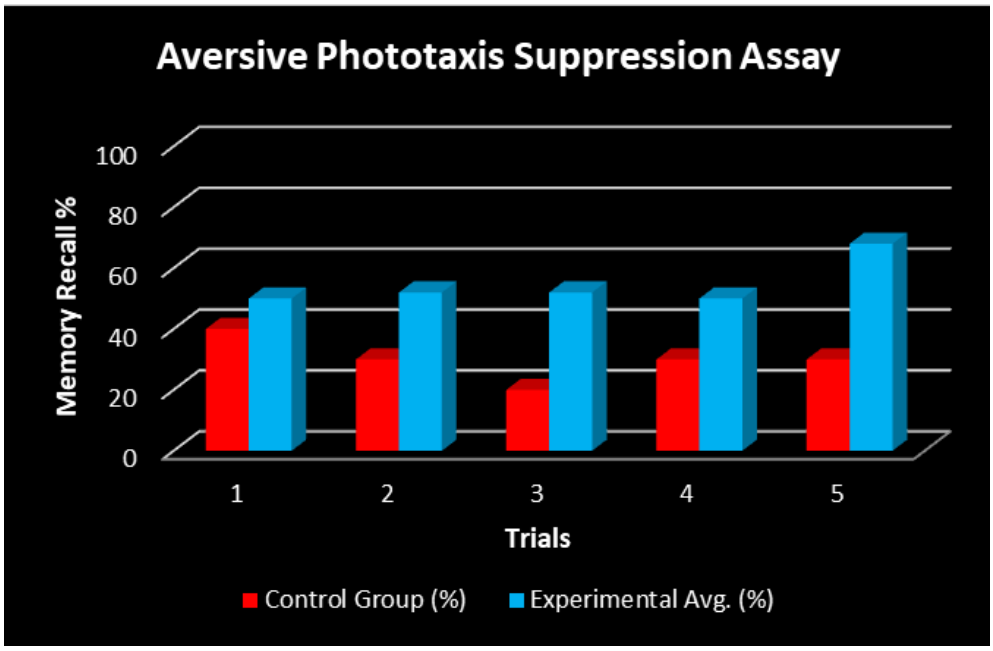
The flies were then split into six groups. The first was the control group, and the other five were the experimental groups. The control group was then trained to avoid the sucrose solution. This was done by exposing the flies to the sucrose solution while the T-maze was being shaken for one minute. After ten minutes, the flies were reintroduced into the T-maze and given ten seconds to choose which chamber they would go into, the one with the sucrose or the one without sucrose. The number of flies that failed to walk into the sucrose chamber was recorded. This number was divided by the total number of flies to provide the passing rate. This same procedure was used for the experimental groups with some slight differences. For the experimental groups, the training consisted of the flies being exposed to the sucrose solution and the blue light while the T-maze was being shaken for one minute. Then, while the flies were reintroduced to the T-maze, they were once again exposed to the blue light. The

number of flies that failed to walk into the sucrose chamber was recorded. This number was divided by the total number of flies to provide the passing rate for each experimental group.

6 Results

6.1 Wildtype Flies Results

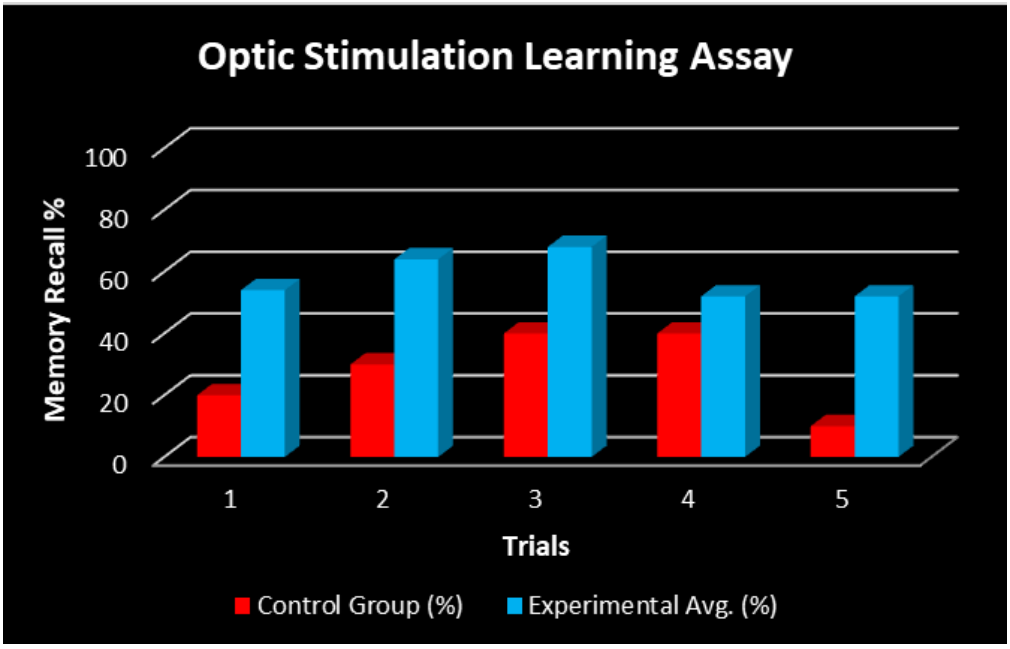
In the Aversive Phototaxis Assay run on the wildtype flies, an average of 24.4 percent more flies in the experimental groups remembered the association between the ethanol scent and the aversive stimulus than the flies in the control group (Table 1). The experimental groups consistently had at least 50 percent more flies avoiding the T-maze chamber with ethanol. Though flies in the experimental group had varying percentages of flies who learned from the training and avoided the ethanol, they consistenly performed better than the control group.



Graph 1: Wildtype Aversive Phototaxis Suppression Assay Results

In the Optic Stimulation Aversive Learning Assay, the control group did not perform as well as the experimental groups. The control group flies had a low performance index, with an average of 28 percent of flies avoiding the sucrose solution, indicating that they remembered the least from training. The flies in the experimental groups constistently avoided the blue light,

associating it with the shaking aversive stimulus. Throughout all the trials, the experimental group flies had an average of 30 percent more flies avoiding the sucrose chamber than the control group.

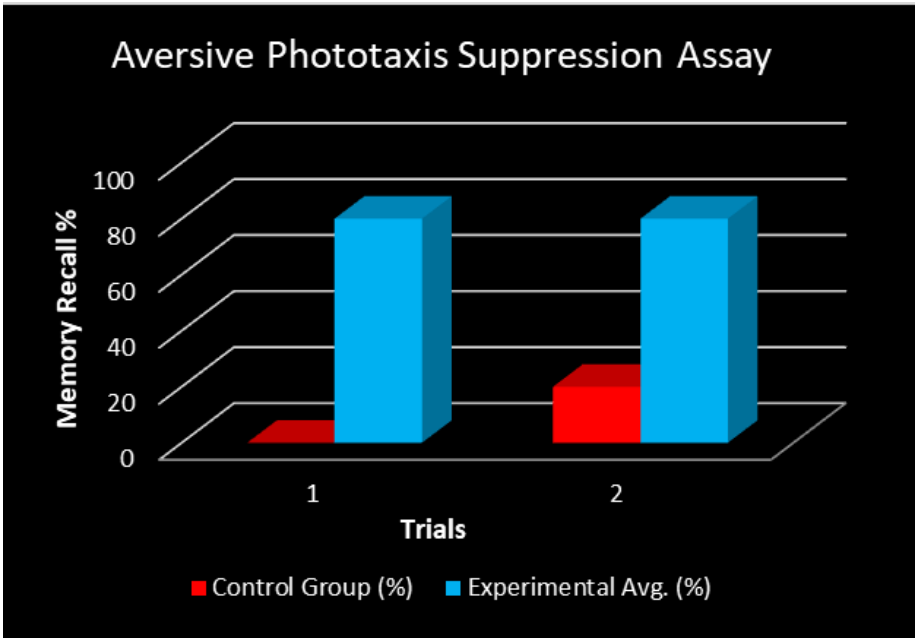


Graph 2: Wildtype Optic Stimulation Aversive Learning Assay Results

6.2 APP Flies Results

In the Aversive Phototaxis Assay, an average of 70 percent more flies in the experimental groups were able to remember that the ethanol scent was associated with the aversive stimulus than the flies in the control group (Table 1). The experimental groups consistently had at least 60 percent of the flies avoiding the T-maze chamber with ethanol. The flies in the

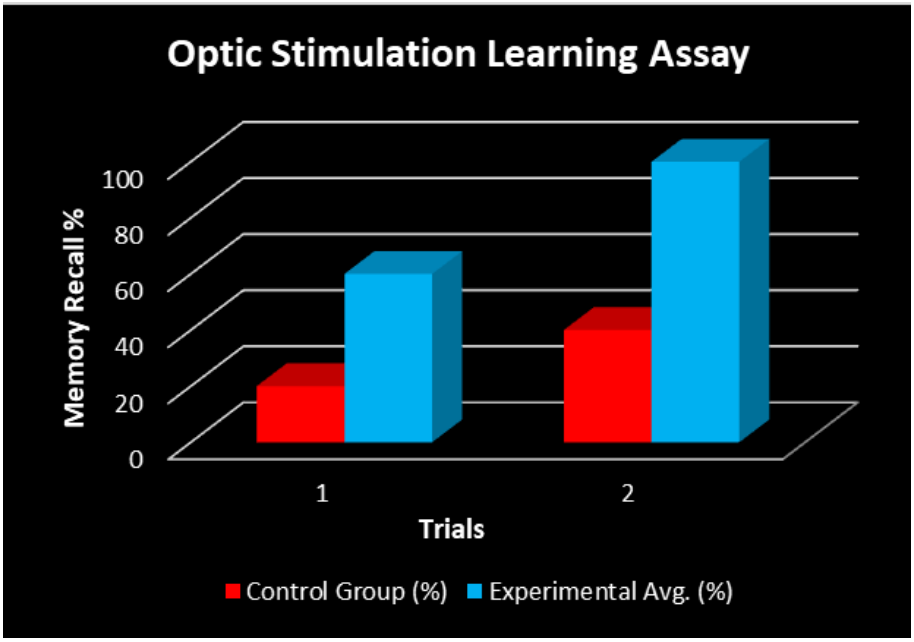
experimental group had different percentages of flies who learned from the training and avoided the ethanol, but they consistenly showed better results than the control group.



Graph 3: APP Flies Aversive Phototaxis Suppression Assay Results

In the Optic Stimulation Aversive Learning Assay, the control group also performed worse than the experimental groups. The control group flies had the lowest performance index, with an average of 30 percent avoiding the sucrose chamber, indicating that they remembered the least from training. The flies in the experimental groups constistently avoided the blue light, associating it with the shaking aversive stimulus. Through all the trials, the flies in the

experimental group had an average of 50 percent more flies avoiding the sucrose solution than the control group.



Graph 4: APP Flies Optic Stimulation Aversive Learning Assay Results

7 Conclusions

The hypothesis that optic and olfactory sensory stimulations enhance memory recall was supported by the results of the experiment. When the *Drosophila melanogaster* were exposed to the light stimulus or the ethanol odor stimulus during the training period, the percentage of flies that learned and remembered the training significantly improved. In the aversive phototaxis suppression assay, the odor stimulant was shown to significantly affect the memory recall in the wildtype flies. In the optic stimulation aversive learning assay, the optic stimulant was also shown to significantly affect the memory recall in the flies. The average number of flies that successfully responded to training in groups exposed to optic, olfactory, or no memory enhancement technique were different, with the optic stimulation increasing the memory recall the most (28 percent memory recall increase). The olfactory stimulation also increased the memory recall in the wildtype flies (24.4 percent memory recall increase) but was not as effective as the optic stimulation. These differences were analyzed using a t-test and found to be statistically significant (Appendix B).

When the aversive phototaxis suppression assay was run using the *Drosophila* expressing the APP gene, the groups given the odor stimulant had a significant increase in memory recall. The optic stimulation aversive learning assay yielded similar results with the groups given the optic stimulant experiencing an increase in memory recall. The average number of APP flies that successfully responded to training in groups exposed to optic, olfactory, or no memory enhancement technique were different, with the olfactory stimulation increasing the memory recall the most (70 percent memory recall increase). The optic

stimulation also increased the memory recall in the APP flies (50 percent memory recall increase) but was not as effective as the optic stimulation. These differences were analyzed using a t-test and found to be statistically significant (Appendix B).

This research can be used for development of cheap and accessible Alzheimer's therapies. In order for these results to be properly integrated into an Alzheimer's therapy, more testing would need to occur to confirm that the stimuli have similar effects with humans as they do with the model organisms. The results are currently being implemented into a reminder system for the elderly and dementia patients, with an optic stimulus being used as the reminder.

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9 Appendix

9.1 Appendix A: Limitations and Assumptions

Limitations:

1. Dr. Duffy is not always available for supervision throughout the week, limiting the possible time to work with the flies
2. Flies take about three weeks to arrive by mail, and then they take two weeks to produce offspring ready for experimentation. This limits the amount of time to actually run the experiments.
3. The sensory stimulations cannot be tested on humans so it is unknown whether the effects will be the same as they are with *Drosophila*.

Assumptions:

1. *Drosophila* model organisms will be representative of humans with Alzheimer's disease.
2. Assays used will accurately test for memory recall.
3. The behavior of the sample size is representative of all flies.
4. The fly population is not contaminated.
5. All offspring from the Gal4/UAS cross would express the APP gene.

9.2 Appendix B: Data

Aversive Phototaxis Suppression Assay Results								
Test #	Control Group	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt Avg. (%)	p-value
1	40%	30%	50%	70%	60%	40%	50%	0.000396
2	30%	80%	40%	70%	30%	40%	52%	
3	20%	60%	50%	50%	30%	70%	52%	
4	30%	60%	30%	50%	70%	40%	50%	
5	30%	50%	70%	70%	60%	90%	68%	

Table 2: Wildtype Aversive Phototaxis Suppression Assay Data

Optic Stimulation Learning Assay Results								
Test #	Control Group	Expt 1	Expt 2	Expt 3	Expt 4	Expt 5	Expt Avg. (%)	p-value
1	20%	40%	70%	50%	60%	50%	54%	0.001052
2	30%	60%	60%	50%	70%	80%	64%	
3	40%	60%	80%	60%	90%	50%	68%	
4	40%	50%	40%	70%	50%	50%	52%	
5	10%	60%	50%	30%	50%	70%	52%	

Table 3: Wildtype Optic Stimulation Aversive Learning Assay Data

Aversive Phototaxis Suppression Assay Results				
Test #	Control Group	Expt 1	Expt Avg. (%)	p-value
1	0%	80%	80%	0.009902
2	20%	80%	80%	

Table 4: APP Flies Aversive Phototaxis Suppression Assay Data

Optic Stimulation Learning Assay Results				
Test #	Control Group	Expt 1	Expt Avg. (%)	p-value
1	20%	60%	60%	0.025658
2	40%	100%	100%	

Table 5: APP Flies Optic Stimulation Aversive Learning Assay Data

9.3 Appendix C: Literature Notes

Journal 1

Source Title	Simple model systems: a challenge for Alzheimer's disease
Source citation	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3388466/
Source type	Journal article
Keywords	Age, Neurodegenerative disease, Animal model, Misfolding, Protein aggregation
Summary	This journal focuses on multiple types of invertebrate model organisms that are being used for studies on Alzheimer's. It delves into the pros and cons for each organism.
Reason for Interest	I am thinking about having some model organisms for my experiments to see how organisms that have Alzheimer's disease react to certain sensory stimulations.

Notes	- C. Elegans
	  - free-living nematode
	  - lives in temperate soil environments
	  - transparent
	  - allows for easy study of organism nervous system
	  - short lifespan
	  - "allows both rapid construction of different transgenic models and quick assessment of the experimental interventions"
	  - Plenty of research with C. Elegans
	  - Lots of background info
	- Danio Rerio
	  - Transparent
	  - Similar organization of nervous system
	  - Similar division of nervous system to humans
	  - Contains neuronal populations that have a direct relevance to neurodegenerative diseases
	- Drosophila melanogaster
	  - Main sensory inputs
	  - Visual / Optic
	  - Olfactory

	- Brain containing 200,000 neurons - Neurons are similar to those of humans
Questions	

Journal 2

Source Title	Olfactory modulation of colour working memory: How does citrus-like smell influence the memory of orange colour?
Source citation	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0203876
Source type	Journal article
Keywords	Working memory, vision, memory, smell, odorants, color vision, attention
Summary	This journal focuses on a study which tested how citrus smell would help participants to recall certain memories which were related to the color orange.
Reason for Interest	I am looking into switching my project to how different senses help for memory recall and I wanted to see different processes that are used for experimentation.

Notes	- Visual sensations can modulate olfactory sensations very often
	- Olfactory sensations are processed in the limbic region
	  - Same as memories and emotion
	- Olfactory sensations can sometimes modulate visual sensations when inputs are associated
	- Olfactory sensations can produce pleasant or unpleasant sensations
	- It may be possible to manipulate connection between visual and olfactory senses to retrieve memories associated to specific colors
	- Olfactory sensations have stronger connection to emotions
	- Participants asked to indicate which colors are most associated with particular odors
	- Olfactory sensations presented with a decanal
	  - Squalene oil was put into a water bath of 35°C
	  - Placed in a box with a hole
	  - Allowed participant to smell out of box
	- No significant ratio found with visual and olfactory association

Questions	

Journal 3

Source Title	Music therapy and Alzheimer's disease: Cognitive, psychological, and behavioural effects
Source citation	https://www-sciencedirect-com.ezproxy.wpi.edu/science/article/pii/S217358081730072X
Source type	Journal article
Keywords	Music therapy, Alzheimer disease, Cognition, Neuropsychiatric symptoms
Summary	This journal focuses on a study which tested how music therapy would affect Alzheimer's patients
Reason for Interest	I wanted to see the data of past experiments on different sensory stimulation to affect Alzheimer's
Notes	- Musical preferences were analyzed through a questionnaire in order to rule out music genres that would upset the participants - No headphones, music played through stereo system - 2 weekly sessions of music therapy for 45 minutes each - Cognitive assessment at halfway mark in therapy and once study was over - Music therapy significantly increased MMSE scores of participants - Demonstrated increase in orientation, language, and memory - Music therapy lessoned symptoms of the neuropsychiatric diseases - Overall, the therapy helped to improve cognitive function

Questions	- What types of music were used?
	  - Specific genres?
	  - Does the music need to be enjoyable for the therapy to be effective?

Journal 4

Source Title	A new era for understanding amyloid structures and disease
Source citation	https://www.nature.com/articles/s41580-018-0060-8
Source type	Journal article
Keywords	Amyloid, Cross-B, Chaperones, Protofilament, Subunits, Amyloidoses, Prion, Age of Onset, Haemodialysis, Phase Separation, Intrinsically Disordered Proteins, Native Protien, Fibril Load, Crossover
Summary	This journal focuses on the cross-β structures discovered in the atomic structure of amyloid fibrils which are commonly associated with pathological conditions which are associated with ageing.
Reason for Interest	I wanted to learn more about amyloid fibrils due to their association with Alzheimer's disease

Notes	- Atomic structure of amyloid fibrils was unknown until now
	- Consists of many cross-β structures
	  - Much more intricate than expected
	  - Amyloid fold -> ladder of stacked β-strands
	- Amyloid fibrils
	  - Hierarchical structures
	  - β-strands form protofilaments which pack together to form amyloid fibrils which form plaques
	- Studies into amyloid structure can help with developing therapy
	  - So far, no therapeutics have reduced amyloid formation
	  - Increased resolution of amyloid structures will help with understanding how fibrils affect the cellular environment

Questions	

Journal 5

Source Title	Drosophila Amyloid Precursor Protein-Like Is Required for Long-Term Memory
Source citation	http://www.jneurosci.org/content/31/3/1032.full
Source type	Journal article
Keywords	
Summary	This journal focuses on the effects of the APPL expression in drosophila melanogaster.
Reason for Interest	Talks about specific fly keeping and might be able to help me find more information on a procedure

Notes	- Flies
	  - Raised on standard medium at 18°C with 60% humidity in a 12 h light/dark cycle
	  - Appl-42673 RNAi line (42673) was obtained from the Vienna Drosophila RNAi Center (Vienna, Austria)
	  - Flies were kept on RU486-containing medium (RU) (SPI-Bio) for 2 d before conditioning and also for 24 h after (when memory was tested at 24 h)
	- Behavioral analysis
	  - Olfactory memory tested
	  - Trained using conditioning protocols described in Pascual and Préat (2001)
	  - Odors were diluted in paraffin oil
	  - Memory tests were performed with a T-maze apparatus. Flies could choose for 1 min between two arms, each delivering a distinct odor. An index was calculated as the difference between the numbers of flies in each arm divided by the sum of flies in both arms. For odor avoidance tests after electric shock and response to electric shock, flies were treated as described by Pascual and Preat (2001).

Questions	

Journal 6

Source Title	Localization of Long-Term Memory Within the Drosophila Mushroom Body
Source citation	http://science.sciencemag.org/content/294/5544/1115?ijkey=3ef867ac5ce7c63fec2c7cdee3a76ff4d4515b4b&keytype2=tf_ipsecsha
Source type	Journal article
Keywords	
Summary	This journal focuses on substructures of the Drosophila brain involved in olfactory and short-term memory to see their effect on long-term memory
Reason for Interest	Learn specific approaches to the procedure

Notes	- The ala mutant was trained to associate an odor with electric shocks by using three different experimental paradigms
	  - a single training cycle protocol to induce short-term memory
	  - an intensive spaced conditioning protocol, consisting of 10 individual training sessions with a 15-min rest interval between each session, to induce long-term memory
	  - massed conditioning protocol, consisting of 10 consecutive training sessions without rest, to induce 24-hour memory but not protein-synthesis dependent LTM

Questions	

Journal 7

Source Title	Bloomington Drosophila Stock Center: Working with Drosophila Stocks
Source citation	https://bdsc.indiana.edu/information/fly-culture.html
Source type	Care Plan for Drosophila melanogaster
Keywords	
Summary	This describes how to maintain Drosophila melanogaster stocks provided by their lab.
Reason for Interest	Learn about how to care for the fruit flies when I conduct my experiments
Notes	- Generation time (from egg to adult) is approximately: 7 days at 29°C, 9 days at 25°C, 11 days at 22°C, 19 days at 18°C - Stocks kept at room temperature should be transferred to fresh food every 20 to 30 days - keep room temperature backups of stocks maintained at low temperature for the first two or three transfers in case the stocks do poorly - Bottles or vials are tapped on the pounding pad to shake flies away from the plug, the plug is rapidly removed, and the old culture inverted over a fresh bottle or vial. Flies are tapped into the new vessel, or some shaken back into the old one, as necessary, and the two are rapidly separated and re-plugged.
Questions	

Journal 8

Source Title	Light-Induced Activation of Distinct Modulatory Neurons Triggers Appetitive or Aversive Learning in Drosophila Larvae
Source citation	https://www.sciencedirect.com/science/article/pii/S0960982206018549
Source type	Journal Article
Keywords	
Summary	This journal focuses on which neurons attribute positive or negative stimulus to certain odors in Drosophila.
Reason for Interest	Learn more about procedures using olfactory and optic stimulation.

Notes	- Five larvae placed in center of cell-culture dish (3.5 cm diameter)
	  - Dish placed in a dark box and half filled with 1% agarose
	- Conditional stimuli - 1 µl of either 3-octanol or n-amyl acetate diluted 1:4 in paraffin oil
	  - Attached to center of lid of dish
	- Appetitive stimulus: agarose contained 2 M fructose
	- Aversive stimulus: agarose contained 3 M NaCl
	- When appetitive stimulus was replaced with light
	  - dish placed under an upright wide-field fluorescence microscope
	- Larvae exposed to one odor (CS+) with the reinforcing stimulus for 5 min
	- Then exposed to the other odor without any reinforcing stimulus (CS–)
	- Training procedure repeated 3 times then larvae were “transferred to the center of an agarose-containing cell-culture dish in which 1 µl of the two odors was spotted into the lids of 100 µl PCR tubes placed at opposite sides of the

	dish, and the dish was placed into a dark box." - "Appetitive learning was tested on dishes containing 1% pure agarose, and aversive learning was tested on dishes containing 1% agarose with 3 M NaCl. After 1 min, the number of larvae (n) on either side of the dish was counted. Learning indices for each trial were calculated as: (n (CS+) − n (CS−))/(n (CS+) + n (CS−)). For statistics, the Wilcoxon matched-pairs signed-ranks test and Bonferroni correction were used."
Questions	

Journal 9

Source Title	GAL4 System in *Drosophila*: A Fly Geneticist's Swiss Army Knife
Source citation	Duffy, J. B. (2002). GAL4 system in drosophila: A fly geneticist's swiss army knife. Genesis, 34(1), 1-15. doi:10.1002/gene.10150
Source type	Journal Article
Keywords	
Summary	This journal describes the processes of the GAL4/UAS system of gene expression in *Drosophila melanogaster*
Reason for Interest	Learn more about how to use the GAL4/UAS system to express the genes of interest.

Notes	- Gal4 encodes a protein of 881 amino acids
	- Not limited to *Drosophila*
	  - Can function in variety of systems to activate transcription from the UAS promoter
	- Responder lines mated to flies expressing GAL4 in a particular pattern, termed the driver
	- Depended on temperature
	- Minimal activity is present at 16 degrees (C)
	- 29 degrees (C) provides a balance between maximal GAL4 activity
	  - Has minimal effects on fertility and viability due to growth at high temperature
	- Specific reporter genes can be used to identify whether gene is being properly expressed
	  - UAS-Green Fluorescent Protein (GFP) can be used to tag the gene and viewed under a microscope to detect gene expression
	- Powerful tool for misexpression studies
	- GAL4 drivers can be extended to *Drosophila* cell culture (wide scope)
	  - With cell culture responders can

	be rapidly tested for expression and/or function - Limitation: Current lack of responder constructs and lines for all genes in the genome - "Benign" reporters can be used for identification and characterization of mutations affecting a specific developmental process
Questions	

Journal 10

Source Title	A single pair of neurons links sleep to memory consolidation in Drosophila melanogaster
Source citation	https://elifesciences.org/articles/03868#sthash.DLYOflYT.dpuf
Source type	Journal Article
Keywords	
Summary	This journal describes the connection between sleep and memory in fruit flies.
Reason for Interest	Learn more about mushroom bodies (responsible for memory and associative learning)
Notes	- DPM neurons increase sleep with the release of GABA onto wake-promoting mushroom body neurons - Downregulation of α'/β' GABAA and GABABR3 receptors results in sleep loss - Sleep deprivation following an associative learning task impairs consolidated memory in *Drosophila* - dorsal paired medial (DPM) neurons are critical to memory consolidation in *Drosophila melanogaster* - MBs are a sensory integration center in the insect brain - Shown to be critical nodes for attention and arousal - Long-term memory storage in mammals is believed to involve a transfer of information from one brain region to another - DPM neurons are inhibitory - Study suggests DPMs probably don't participate directly in the excitatory arm of a recurrent feedback loop - DPM-APL network could represent an analogous set of neurons in *Drosophila* which function to coordinate the gating and transfer of different memory stages between different sub-circuits within the

	MBs - wake-promoting phenotype of MB α'/β' neurons as well as a shared temporal role in memory consolidation suggest these neurons could be the targets of sleep-promoting DPM GABA release
Questions	