The Effect of Natural and Chemical Remedies on Wildtype and Mutant Antennapedia *Drosophila melanogaster*

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Abstract

Anosmia, the inability to perceive odor, is associated with olfactory nerve damage and culminates in abated food enjoyment and compensatory eating habits, thereby increasing medical risks such as high blood pressure, heart attack, and stroke. There is a demand for natural anosmia treatments over their chemical-based counterparts, as the latter are associated with numerous detrimental side effects. In this work, the olfactory systems of *Drosophila melanogaster* were studied, specifically chosen for their highly-advanced ability to trace odor molecules at low concentrations. A mutation in the Antennapedia gene in *Drosophila* results in a secondary pair of legs substituting the antennas. As studies regarding the mutation in relation to olfactory systems have been limited, this study explored the antennas' olfactory role. Further, it sought to compare natural remedies-- coffee, garlic, and a combination thereof-- and chemical remedies-- lipoic acid--in order to determine whether the natural remedies tested can be used as a substitute for current anosmia treatment. Similar to previous olfaction studies, olfactory avoidance experiments were performed to study olfactory efficiency, which refers to how capable an organism is to detect and respond to a scent. Independent, two-tailed t-tests were conducted between the mean reaction times of groups in the following scenarios: (i) control untreated group vs. treated, (ii) chemically vs. naturally treated, (iii) before-treatment vs. after-treatment, and (iv) mutant vs. wildtype. The p-values were found to be 0.00147, 0.01015, 0.25172, 0.01989, and 2.2313 E-09, which, compared with an alpha level of 0.05, resulted in concluding a significant difference in four out of the five trials between mutant and wildtype reaction times, supporting that wildtype *Drosophila* exhibit better-developed olfactory systems than the mutants. Data revealed a significant difference between reaction times of every treatment and control; however there was no significant difference between reaction times of groups treated by natural remedies, in comparison to one another. Data supports the hypothesis of lipoic acid and coffee reducing the mean reaction times and increasing in performance indices to the greatest extents; however, the results support the use of all natural treatments tested to create safer anosmia treatments.

Introduction

Anosmia, the inability to perceive odor, is commonly attributed to nasal conditions, sinus infections, or exposure to certain chemicals in temporary cases (*Smell Disorders*, n.d.). As olfactory nerve damage is oftentimes caused by facial and skull injuries, brain and head trauma result in permanent anosmia in severe cases. Consequences include abated enjoyment of food, which typically manifests itself in the form of modified compensatory eating habits comprised of excess ingredients such as salt and sugar. Excessive use of such is shown to have a strong correlation with medical conditions including cardiovascular diseases, high blood pressure, and diabetes (C. W., 1989) . Smell disorders are frequently indicative of Parkinson's disease, Alzheimer's disease, multiple sclerosis, hypertension, and obesity (C. W., 1989) .

Hyposmia is a reduced ability to smell, and anosmia is a loss of the ability to smell. In a study conducted on 23 patients with hyposmia or anosmia, 26% showed a moderate increase in olfactory function and 35% showed a remarkable increase in olfactory function after orally consuming alpha-lipoic acid at 600 mg/day for 4.5 months (T. H., 2002) . Half of the anosmia patients improved to hyposmia, and 5 of 19 hyposmic patients developed a normal sense of smell (T. H., 2002) . Contemporary treatments of anosmia are pharmaceutical drugs, long-term antibiotics, surgery, antidepressants, and steroids (Malik, 2014) . A pharmaceutical drug known as alpha-lipoic acid has been shown to enhance olfactory senses.

Despite their effectiveness, current treatments are not only accompanied by dangerous side effects, but are also expensive. For this reason, there is a pressing demand for natural remedies to provide a safer alternative for improving sense of smell. For years, researchers have sought to promote regeneration of sensory nerve cells and understand associations between smell disorders and changes in diet among individuals with illnesses (*Treatment for Anosmia*, 2015) . Olfactory organs, those involved with detecting odors, are most stimulated by strong odors such as coffee and garlic (Dorsi, Yaser&Sabeghi, Maryam, 2007).

Olfactory systems of vertebrates and invertebrates consist of olfactory receptor neurons (ORNs), located on membranes of the cilia, that allow for the detection of odors (Laissue, P. P., & Vosshall, L. B., n.d.) . Once an odorant dissolves into the olfactory epithelium, it binds to an ORN and the organism responds to the odor through repulsion or attraction (Laissue, P. P., & Vosshall, L. B., n.d.). The olfactory systems of insects are highly developed in order to identify odors to avoid predators, detect food, and mate. As complex olfactory systems are crucial to their survival, insects have the ability to detect and differentiate between thousands of odors. The antennae and maxillary palps of insects are primarily used for olfaction, as they comprise heavily of ORNs (Laissue, P. P., & Vosshall, L. B., n.d.).

Researchers have extensively studied the olfactory systems of *Drosophila melanogaster* as they are capable of easily tracing odor molecules at concentrations much lower than those detectable by other organisms (Wang, J. W., 2009). *Drosophila* are used commonly to study the olfactory systems of humans for reasons twofold: their simplicity and similarity in disease manifestation. Specifically, *Drosophila*, colloquially referred to as fruit flies, are easy to maintain due to their small, fully-mapped genome size and short life cycle. In addition, sixty percent of the genes involved in human diseases have a homologue in *Drosophila*. Similar to other insect olfactory systems, fruit flies detect odors through the maxillary palp and antennae on their head (Laissue, P. P., & Vosshall, L. B., n.d.). Despite the simplicity of their brains, *Drosophila* have an estimated 2,600 ORNs situated on the 410 olfactory sensilla covering the antenna and 60 olfactory sensilla covering the maxillary palp (Laissue, P. P., & Vosshall, L. B., n.d.). A mutant species of *Drosophila*, however, lacks this abundance of ORNS due to its mutated copy of the Antennapedia (*Antp*) gene that controls thoracic development. One copy of the mutated *Antp* results in the formation of a second pair of legs in place of the antennas.

Prior experiments on *Drosophila* have used olfactory avoidance experiments to quantify the "effectiveness" of the olfactory systems. These experiments are framed around measuring the capability of flies to associate an odor with a negative reinforcer, such as an electric or mechanical shock, to subsequently avoid the scent. Should the olfactory abilities be in ideal condition, the flies should swarm the scent that was not negatively reinforced. In measuring the fraction of the flies whose behavior strayed from this behavior, a quantifying of "olfactory effectiveness" is achieved.

Consider an illustrative example, in which a group of flies is exposed to scents A and B. The flies are first exposed to the former, simultaneously experiencing an electrical shock. They are then exposed to scent B, in which no such negative reinforcer is present.

Afterwards, the flies are exposed to an area containing both scents, wherein the number that swarm A vs. those that swarm B are recorded. The performance index can then be calculated as the fraction of flies that swarmed B, whose value clearly ranges from zero to one, with the higher values indicating a higher efficiency of the olfactory systems. The time flies needed to detect and travel to the scent can additionally be recorded. A decrease in reaction time indicates faster recognition and response to the scent and, thus, better olfactory systems.

The study has far-reaching repercussions not only in the medical field, but additionally in agriculture. Insects serve as a solid foundation of our global environment as they support ecosystems, provide food for animals, and support human life. In order to survive, insects heavily rely on their olfactory systems and, for this reason, the results of this study can be applied to significantly improve this system within insects. As consumers, scavengers, and decomposers, insects play a vital role in the biogeochemical cycling of nutrients as they aerate the soil, improve its retention of rainwater, and enhance soil by redistributing nutrients within the root zone as they burrow and nest in the ground. Furthermore, they prevent the buildup of waste products from large animals and speed up its decomposition; therefore, a lack of insects would result in the accumulation of manure that would render a large portion of landscape unsuitable for agricultural purposes. This experiment seeks to determine treatments to improve the olfactory systems of insects which, in turn, will support the environment and agriculture.

Conditioning experiments have been used to study the efficiency of the olfactory senses of wildtype and mutant *Drosophila* to understand the organs involved with sensing odors. An olfactory avoidance experiment was done on adult *Drosophila* in which the flies were trained to fly towards a scent in the first phase and tested in the second phase.This time, however, the flies were tested by placing them in a T-maze with both scents and recording which scent they flew towards (*Treatment for Anosmia*, 2015) . The study found that the mutant adult flies show a reduction in learning in comparison to the wild type flies (*Treatment for Anosmia*, 2015) .Future studies seek to use imaging and heat-activated channels to activate or inhibit expression of various neurons associated with memory in order to determine their specific functions (*Treatment for Anosmia*, 2015) .

As research on the effect of the Antennapedia mutation on olfaction is limited, this study sought to explore whether the mutation of the *Antp* geneaffects olfactory neuron growth by determining whether there is a significant difference in efficiency of olfactory systems in the mutant and wild type *Drosophila melanogaster*. The study additionally sought to distinguish the effectiveness of natural treatments against their chemical counterparts.

Hypothesis

It is predicted that the Antennapedia mutants will exhibit less developed olfactory systems as the majority of ORNs are typically situated on the antenna of *Drosophila*. Overall, alpha lipoic acid is predicted to best improve olfactory systems; however, from the natural remedies, the coffee is predicted to improve the olfactory systems of the flies to the greatest extent. In this experiment, olfactory avoidance experiments will be performed on wildtype and mutant *Drosophila* using lime juice accompanied by mechanical shock as negative reinforcement as well as apple cider vinegar. Different substances will be added to the foods of the flies as natural treatments-coffee, garlic, and a combination thereof- and chemical treatment- alpha lipoic acid. The olfactory avoidance experiment will be performed and compared to the control group that was not treated with any substance in the food in order to conclude whether the treatment enhanced or reduced olfactory senses. Based on the results, this experiment can be used as a basis to creating treatments for anosmia with less side effects and improve insects' abilities to sense smell in order to survive and continue serving as a fundamental part of the ecosystem.

Materials and Methods

Item	Quantity	Purpose
Wild type& Mutant Antennapedia *Drosophila melanogaster*	5 tubes of each fly type	The *Drosophila* serve as the model organism of the study used to test the natural and chemical treatments on.
Apple Cider Vinegar and Lime Juice	1 bottle of each liquid	Both juices are used in the olfactory avoidance experiment as the scents.
Cotton Pads	100	The apple cider vinegar and lime juice are pipetted onto these pads, which are used to cover the fly tube to expose the organisms to the scent.
Disposable Pipet	Pack of 100	The pipet is used to transfer the apple cider vinegar and lime juice from the bottle to the cotton pads.
Black Coffee Grounds and Garlic	1 pack of coffee grounds and 1 piece of garlic	The coffee grounds and garlic are used as the natural treatments to be added to the fly cornmeal food.
Alpha-lipoic Acid Tablets	1 bottle	The alpha-lipoic acid tablets are used as the chemical treatment to be added to the fly cornmeal food.
Fly Cornmeal and Dry Yeast	1 bag of cornmeal and 5 packs of dry yeast	The fly cornmeal and dry yeast are used as the food supply for the organisms.
Fly Vials and Fly Vial Plugs	50 of each	The fly vials and plugs are used to store the organisms.
Vortex	1	The vortex is used to mechanically shock the flies during the olfactory avoidance experiment.

3.1 Experimental Design Components

Independent Variables	Fly type: Wild type *Drosophila* or Mutant Antennapedia *Drosophila* Food treatment: No treatment, coffee grounds, garlic shavings, combined coffee grounds and garlic shavings, and alpha-lipoic acid powder.
Dependent Variables	Performance indices Mean reaction times
Control	Untreated Wild type and Mutant *Drosophila melanogaster*
Constants	Number of flies being used (25 flies of each fly type per trial) Number of flies in each treatment group (5 flies) Environment Temperature of classroom Time of exposure to apple cider vinegar and lime juice Frequency and time duration of mechanical shock Concentrations of apple cider vinegar and lime juice
Total trial	5 trials

3.2 Olfactory Avoidance Experiments

In order to condition the first group of wildtype flies, apple cider vinegar and lime juice were pipetted onto separate cotton pads. The fly vial plug was removed and replaced with the cotton pad containing the lime juice. This exposure to the scent was accompanied by a mechanical shock by vortexingthe vial 10 times for 2 seconds each at 4 second intervals. After the mechanical shock, the fly vial plug was inserted to replace the cotton pad and there was a 1 minute rest period without any odor or shock. After one minute, the fly vial plug was again removed and replaced with the cotton pad containing apple cider vinegar. The flies were exposed to this scent for one minute, and then the fly plug was inserted to replace the cotton pad. The flies were then transferred to a tube containing the apple cider vinegar cotton pad on one end and lime juice cotton pad on opposite end. A video was recorded using a phone camera to observe how many of the five flies travelled to each scent and the time taken. This procedure was repeated five times for each fly groups for both the mutant and wildtype flies.

3.3 Treated the flies

In order to treat the flies, coffee grounds, garlic shavings, a combination of garlic shavings and coffee grounds, or alpha-lipoic powder was added to regular cornmeal fly food. Five wild type flies were left in the original vial containing the untreated food to serve as the control. The remaining 20 flies were split into four groups of 5 flies and transferred to each of the four vials labeled with the treatment: control, coffee, garlic, lipoic acid, and combination. After one week, the olfactory avoidance experiment described in 3.3 was performed for each of the normal and mutant groups.

3.4 Statistical tests

Null Hypothesis (H_0)	***Alternate Hypothesis (H_1)***
There is no significant difference in the mean reaction times before and after treatment in mutant and wildtype Drosophila.	*There is a significant difference in the mean reaction times before and after treatment in mutant and wildtype Drosophila.*
There is no significant difference in the original mean reaction times of wildtype and mutant Drosophila.	*There is a significant difference in the original mean reaction times of wildtype and mutant Drosophila.*
There is no significant difference in the mean reaction times of wildtype Drosophila group with different treatments.	*There is a significant difference in the mean reaction times of wildtype Drosophila group with different treatments.*
There is no significant difference in the mean reaction times of wildtype Drosophila groups with no treatment and treatment.	*There is a significant difference in the mean reaction times of wildtype Drosophila groups with no treatment and treatment.*

In order to analyze the data, the performance indices (PI) were calculated for the flies before and after treatment.The standard deviation of the mean reaction times of each group was found to show the variance in data of the times it took for the flies to react. Two-tailed, independent t-tests were performed to conclude if there was any significant difference between the mean reaction times of the following situations: (i) before and after each treatment (ii) Antennapedia and wild type groups (iii) each treatment and the control (iv) each treatment. -

Results

Table 1 Mean Reaction Times & Standard Deviation of Wildtype Flies

Trial	Group	Pre-treatment (Sec)	Post-treatment (sec)	Percent Change in Reaction Time (%)
1	Overall	49.4 ± 10.9	31.6 ± 12.3	-36.0
	Control Group 1	46.4 ± 15.6	43.8 ± 17.1	-5.6
	Coffee Group 2	48.2 ± 8.0	28.4 ± 11.3	-41.1
	Garlic Group 3	58.4 ± 10.4	32.2 ± 7.9	-44.9
	Combined Group 4	48.2 ± 11.4	28.8 ± 8.3	-40.2
	Alpha-Lipoic Acid Group 5	45.8 ± 6.1	25.0 ± 9.2	-45.4
2	Overall	52.7 ± 5.8	27.1 ± 8.1	-48.6
	Control Group 1	48.0 ± 4.8	43.6 ± 11.9	-9.2
	Coffee Group 2	51.2 ± 6.6	22.0 ± 3.2	-57.0
	Garlic Group 3	55.0 ± 3.7	30.2 ± 8.5	-45.1
	Combined Group 4	52.8 ± 5.1	35.8 ± 9.3	-32.2
	Alpha-Lipoic Acid Group 5	56.6 ± 6.1	22.0 ± 3.7	-61.1
3	Overall	52.4 ± 9.7	40.0 ± 9.2	-23.7
	Control Group 1	54.0 ± 8.7	52.6 ± 7.7	-2.6
	Coffee Group 2	51.0 ± 7.6	38.6 ± 7.7	-24.3
	Garlic Group 3	52.8 ± 15.7	40.2 ± 6.6	-23.9
	Combined Group 4	48.6 ± 5.4	35.6 ± 4.0	-26.7
	Alpha-Lipoic Acid Group 5	55.6 ± 11.1	33.0 ± 6.7	-40.6
4	Overall	53.1 ± 6.9	39.1 ± 7.7	-26.4

	Control Group 1	46.6 ± 9.5	46.8 ± 6.2	0.4
	Coffee Group 2	54.6 ± 5.5	36.8 ± 4.1	-32.6
	Garlic Group 3	57.6 ± 3.8	42.2 ± 6.8	-26.7
	Combined Group 4	56.6 ± 3.3	40.8 ± 3.3	-27.9
	Alpha-Lipoic Acid Group 5	50.2 ± 5.6	28.8 ± 3.2	-42.6
5	Overall	47.6 ± 6.2	38.9 ± 9.7	-18.3
	Control Group 1	45.2 ± 5.3	53.2 ± 3.5	17.7
	Coffee Group 2	49.2 ± 8.3	32.2 ± 9.0	-34.6
	Garlic Group 3	47.0 ± 7.3	41.4 ± 4.5	-11.9
	Combined Group 4	50.4 ± 3.8	36.0 ± 4.4	-28.6
	Alpha-Lipoic Acid Group 5	46.2 ± 6.3	31.6 ± 5.9	-31.6

4.1 Mean Reaction Times of Wildtype Drosophila

The mean reaction times with standard deviation of wildtype flies for the five groups per trial are shown in Table 1 before treatment and after treatment. As the mean reaction times of the fly groups varied in the pre-treatment olfactory experiment, the percentage change in reaction time was found and used to compare each treatment instead of solely comparing the after-treatment values based on which values were highest. The greatest percent change in mean reaction time, -61.1%, was observed in Group 5 of trial 2 that had been treated using alpha-lipoic acid. The groups treated with coffee and alpha-lipoic acid exhibited the two greatest percentage change in mean reaction times, with the exception of trial 3 in which the groups treated with the alpha-lipoic acid and the combination of garlic and coffee exhibited the two greatest percentage changes in mean reaction times.

To determine the effect of each treatment on wildtype *Drosophila*, olfactory avoidance experiments were performed for five trials and the mean reaction time was recorded. Figure 1 shows the percentage change in mean reaction times was calculated for each treatment (Graph 1.1). It should be noted that the values on the graph tend to be negative, an indication of a decrease in the mean reaction times. T-tests were performed to determine whether there was a significant difference between: (i) Mean reaction times between wildtype groups before and after each treatment (ii) Difference in mean reaction times between each treatment and the control (iii) Difference in mean reaction times between every treatment. P-values less than 0.05 that indicated a significant difference were indicated on the figure using different symbols. Significant differences were found between the mean reaction times of wildtype flies: (i) before and after treatment for each group besides the control in all trials, with the exception of the garlic treatment group in trials 3 and 5 (ii) in treatment groups and control groups in the alpha-lipoic acid group in every trial, combined group in 3 trials, garlic group in 3 trials, and coffee group in three trials (iii) alpha-lipoic acid group and garlic group in trial 5 as well as alpha-lipoic acid group and combination group in trial trial 2 (iv) coffee group and combined group in trial 2.

Table 2 Mean Reaction Times & Standard Deviation of Mutant Flies

Trial	Group	Pre-treatment (sec)	Post-treatment (sec)	Percent Change in Reaction Time (%)

1	Overall	60.0 ±9.6	52.0 ± 9.0	-13.3
	Control Group 1	61.0 ± 6.3	54.8 ± 9.1	-10.2
	Coffee Group 2	60.6 ± 13.0	49.0 ± 8.6	-19.1
	Garlic Group 3	62.2 ± 4.0	53.4 ± 7.7	-14.1
	Combined Group 4	53.4 ± 13.7	49.6 ± 5.7	-7.1
	Alpha-Lipoic Acid Group 5	63.0 ± 8.3	47.0 ± 12.0	-25.4
2	Overall	58.8 ± 9.7	57.4 ± 8.5	-2.4
	Control Group 1	55.0 ± 14.2	60.0 ± 8.8	9.1
	Coffee Group 2	58.8 ± 4.1	52.2 ± 7.8	-11.2
	Garlic Group 3	59.0 ± 10.3	60.6 ± 11.2	2.7
	Combined Group 4	54.8 ± 10.3	58.2 ± 6.5	6.2
	Alpha-Lipoic Acid Group 5	64.8 ± 7.0	55.8 ± 8.2	-13.9
3	Overall	55.4 ± 6.9	52.4 ± 4.5	-5.4
	Control Group 1	54.6 ± 6.2	54.4 ± 4.1	-0.4
	Coffee Group 2	60.0 ± 5.0	52.8 ± 4.9	-12
	Garlic Group 3	56.4 ± 5.3	54.2 ± 5.1	-3.9
	Combined Group 4	49.6 ± 6.8	48.2 ± 4.4	-2.8
	Alpha-Lipoic Acid Group 5	56.2 ± 8.6	52.6 ± 2.3	-6.4
4	Overall	58.0 ± 7.3	53.8 ± 6.7	-7.2
	Control Group 1	56.4 ± 7.1	57.8 ± 6.0	2.5
	Coffee Group 2	58.0 ± 3.6	54.2 ± 4.7	-6.6
	Garlic Group 3	64.6± 6.2	57.8 ± 5.2	-10.5
	Combined Group 4	54.2 ± 11.6	52.4 ± 6.0	-3.3
	Alpha-Lipoic Acid Group 5	57.0 ± 3.1	47.0 ± 7.2	-17.5
5	Overall	62.0 ± 8.4	56.3 ± 7.2	-9.2
	Control Group 1	66.0 ± 8.2	64.0 ± 10.4	-3.0
	Coffee Group 2	61.8 ± 4.6	55.2 ± 8.7	-10.7
	Garlic Group 3	58.6 ± 14.2	58.6 ± 3.6	0.0
	Combined Group 4	62.4 ± 5.3	54.0 ± 4.9	-13.5
	Alpha-Lipoic Acid Group 5	61.4 ± 8.1	52.6 ± 5.3	-14.3

4.2 Mean Reaction Times of Mutant Drosophila

The reaction time statistics, means, and standard deviations of mutant flies before and after treatment for the five groups per trial are shown in Table 2. As the mean reaction times of the five groups varied in the pre-treatment olfactory experiment, the percentage

change in reaction time was found and used to compare each treatment instead of solely comparing the after-treatment values based on which values were highest. The greatest percent change in mean reaction time, -25.4%, was observed in Group 5 of trial 1 that had been treated using alpha-lipoic acid. The group alpha-lipoic acid exhibited the greatest percentage change in mean reaction times, followed by the group treated with coffee. This is with the exception of trials 4 in which the group treated with the garlic exhibited a 10.5% decrease in mean reaction time whereas the group treated with coffee exhibited a 6.6% decrease. Similarly, in trial 5 the group treated with combination of garlic and coffee exhibited a 13.5% decrease in mean reaction time whereas the group treated with the coffee exhibited a 10.7% decrease in mean reaction time.

To determine the effect of each treatment on mutant *Drosophila*, olfactory avoidance experiments were performed for five trials and the mean reaction time was recorded. Using the mean reaction times of the olfactory avoidance experiments before and after treatment, the percentage change in reaction times was calculated for each treatment and plotted (Graph 1.1). Negative bars on the figure represent a decrease in mean reaction time. T-tests were performed to determine whether there was a significant difference in mean reaction times between wildtype groups before and after each treatment. Based on this, a conclusion of whether each treatment significantly improved or worsened the fruit fly olfactory systems was met. Those trials with p-values less than 0.05indicated significant difference were plotted on the figure. Significant differences were noted in the control, coffee, and alpha-lipoid acid groups of trial 1 as well as the alpha-lipoic acid group of trial 4.

Table 3 Performance Indices of Wildtype Flies

Trial	Group	Pre-treatment Performance Index	Post-treatment Performance Index	Change in Performance Index
1	Overall	0.2	0.6	0.4
	Control Group 1	0.2	0.2	0.0
	Coffee Group 2	0.2	1	0.8
	Garlic Group 3	0.2	0.6	0.4
	Combined Group 4	0.2	0.2	0.0
	Alpha-Lipoic Acid Group 5	0.2	1	0.8
2	Overall	0.1	0.4	0.3
	Control Group 1	-0.2	-0.2	0.0
	Coffee Group 2	0.2	0.6	0.4
	Garlic Group 3	0.2	0.2	0.0
	Combined Group 4	0.2	0.2	0.0
	Alpha-Lipoic Acid Group 5	0.2	1	0.8
3	Overall	0.2	0.7	0.5
	Control Group 1	0.2	0.2	0.0
	Coffee Group 2	0.2	1	0.8
	Garlic Group 3	0.2	0.6	0.4
	Combined Group 4	0.2	0.6	0.4
	Alpha-Lipoic Acid Group 5	0.2	1	0.8
4	Overall	0.2	0.5	0.3
	Control Group 1	0.2	0.2	0.0
	Coffee Group 2	0.2	0.6	0.4
	Garlic Group 3	0.2	0.2	0.0

	Combined Group 4	0.2	0.6	0.4
	Alpha-Lipoic Acid Group 5	0.2	1	0.8
5	Overall	0.3	0.6	0.3
	Control Group 1	0.2	0.2	0.0
	Coffee Group 2	0.2	1	0.8
	Garlic Group 3	0.6	0.6	0.0
	Combined Group 4	0.2	0.6	0.4
	Alpha-Lipoic Acid Group 5	0.2	0.6	0.4

4.3 Effect of Treatments on Performance Indices of Wildtype Drosophila

To determine the effect of each treatment on wildtype *Drosophila*, olfactory avoidance experiments were performed five times and the number of flies that traveled to each scent was recorded. Using this, the performance indices were calculated before and after each treatment and the change in performance indices was plotted (Graph 1.2). Positive bars indicate an improvement in the PI whereas the absence of bars indicates no change in PI.

The performance indices of wildtype flies for the five groups per trial are shown in Table 3 before treatment and after treatment. As the performance index (PI) of the fly groups varied in the pre-treatment olfactory experiment, the change in the PI was found and used to compare each treatment instead of solely comparing the after-treatment values based on which values were highest. The greatest change in PI, 0.8, was observed in the groups treated with coffee and alpha-lipoic acid in trial 1, alpha-lipoic acid in trial 2, coffee and alpha-lipoic acid in trial 3, alpha-lipoic acid in trial 4, and coffee in trial 5. The control group of all five trials exhibited no change in PI. The groups treated with the combined treatment and garlic treatment had changes, ranging from 0 to 4, in their PIs.

Table 4 Performance Indices of Mutant Flies

Trial	Group	T1 Pre-treatment	T1 Post-treatment	Change in Performance Index
1	Overall	0.1	0.1	0
	Control Group 1	-0.2	-0.6	-0.4
	Coffee Group 2	0.2	0.2	0
	Garlic Group 3	0.2	0.2	0
	Combined Group 4	0.2	0.2	0
	Alpha-Lipoic Acid Group 5	0.2	0.6	0.4
2	Overall	0.0	0.1	0.1
	Control Group 1	-0.2	-0.2	0.0
	Coffee Group 2	0.2	0.6	0.4
	Garlic Group 3	-0.2	-0.2	0.0
	Combined Group 4	0.2	0.2	0.0
	Alpha-Lipoic Acid Group 5	0.2	0.2	0.0
3	Overall	-0.1	0.2	0.3
	Control Group 1	0.2	0.2	0.0
	Coffee Group 2	-0.2	0.2	0.4
	Garlic Group 3	-0.2	-0.2	0.0
	Combined Group 4	0.2	0.6	0.4

	Alpha-Lipoic Acid Group 5	-0.6	0.3	0.9
4	Overall	-0.2	0.1	0.3
	Control Group 1	-0.6	-0.6	0.0
	Coffee Group 2	-0.2	0.2	0.4
	Garlic Group 3	-0.2	0.2	0.4
	Combined Group 4	0.2	0.6	0.4
	Alpha-Lipoic Acid Group 5	-0.2	0.2	0.4
5	Overall	0.0	0.2	0.2
	Control Group 1	-0.2	-0.2	0.0
	Coffee Group 2	-0.2	0.2	0.4
	Garlic Group 3	0.2	0.2	0.0
	Combined Group 4	0.2	0.6	0.4
	Alpha-Lipoic Acid Group 5	-0.2	0.2	0.4

4.4 Performance Indices of Mutant Drosophila

To determine the effect of each treatment on mutant *Drosophila*, olfactory avoidance experiments were performed as described in Section 4.3. Using this, the performance indices were calculated before and after each treatment and the change in performance indices was plotted (Graph 1.2).

The performance indices of mutant flies for the five groups per trial are shown in Table 4 before treatment and after treatment. As the performance index (PI) of the fly groups varied in the pre-treatment olfactory experiment, the change in the PI was found and used to compare each treatment instead of solely comparing the after-treatment values based on which values were highest. The greatest change in PI, 0.9, was observed in the group treated with alpha-lipoic acid in trial 3. Every other group exhibited a change in PI between 0 and 0.4. The control group of all trials exhibited no change in PI, with the exception of the 0.4 decrease in PI of the control group in trial 1.

T-tests

Upon conducting the desired t-tests, the following p-values were obtained, where each of the trials was tested with a two-tailed, independent t-test. With an alpha value of .05, the significance of the level was concluded upon determining whether these yielded significant differences, i.e. p-value were found to be <.05 in both. Thus:

Table 5 P-values of Wildtype and Mutant Fly Mean Reaction Times

Trial	***P-value***	***Significance***
1	*0.00147*	*Yes*
2	*0.01015*	*Yes*
3	*0.25172*	*No*
4	*0.01989*	*Yes*
5	*2.2313 E-09*	*Yes*

Table 6 P-Values of Difference in Mean Reaction Times of Mutant and Wildtype Groups Before and After Treatment

Treatment	**Wildtype**	**Significance**	**Mutant**	**Significance**

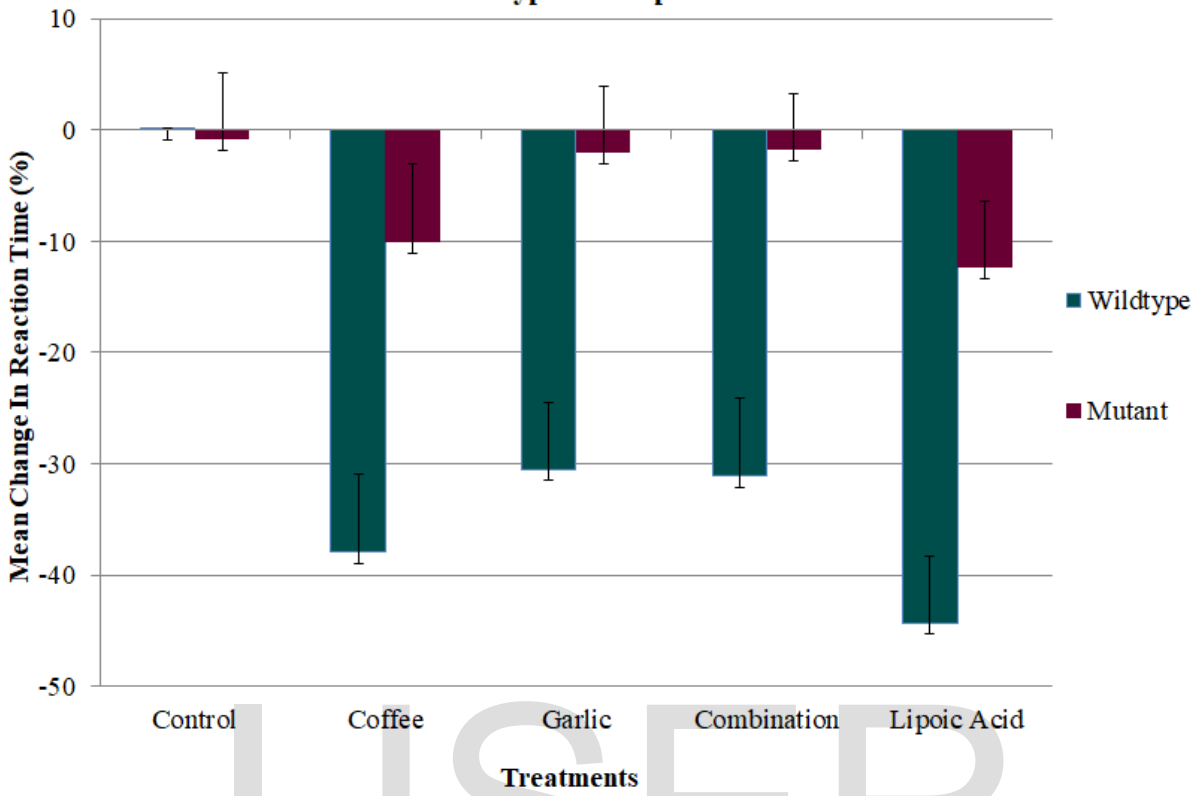
Control	0.63536	No	0.51248	No
Coffee	0.00005	Yes	0.17597	No
Garlic	0.00143	Yes	0.81894	No
Combined	0.00663	Yes	0.56501	No
Alpha-lipoic Acid	0.00050	Yes	0.09850	No

Table 7 P-Values of Difference in Mean Reaction Times of Treated Wildtype Groups vs Control Group

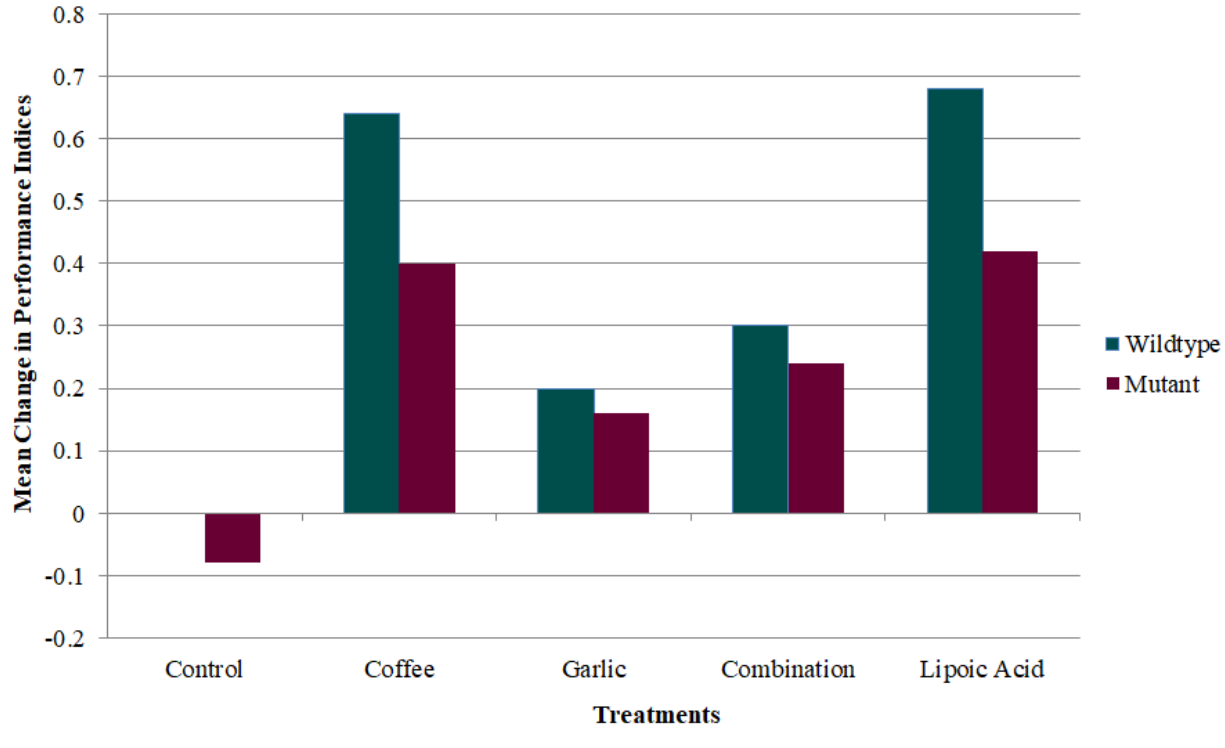
Treatment	P-value	Significance
Combined	0.00514	Yes
Garlic	0.01479	Yes
Coffee	0.00522	Yes
Alpha-Lipoic Acid	0.00165	Yes

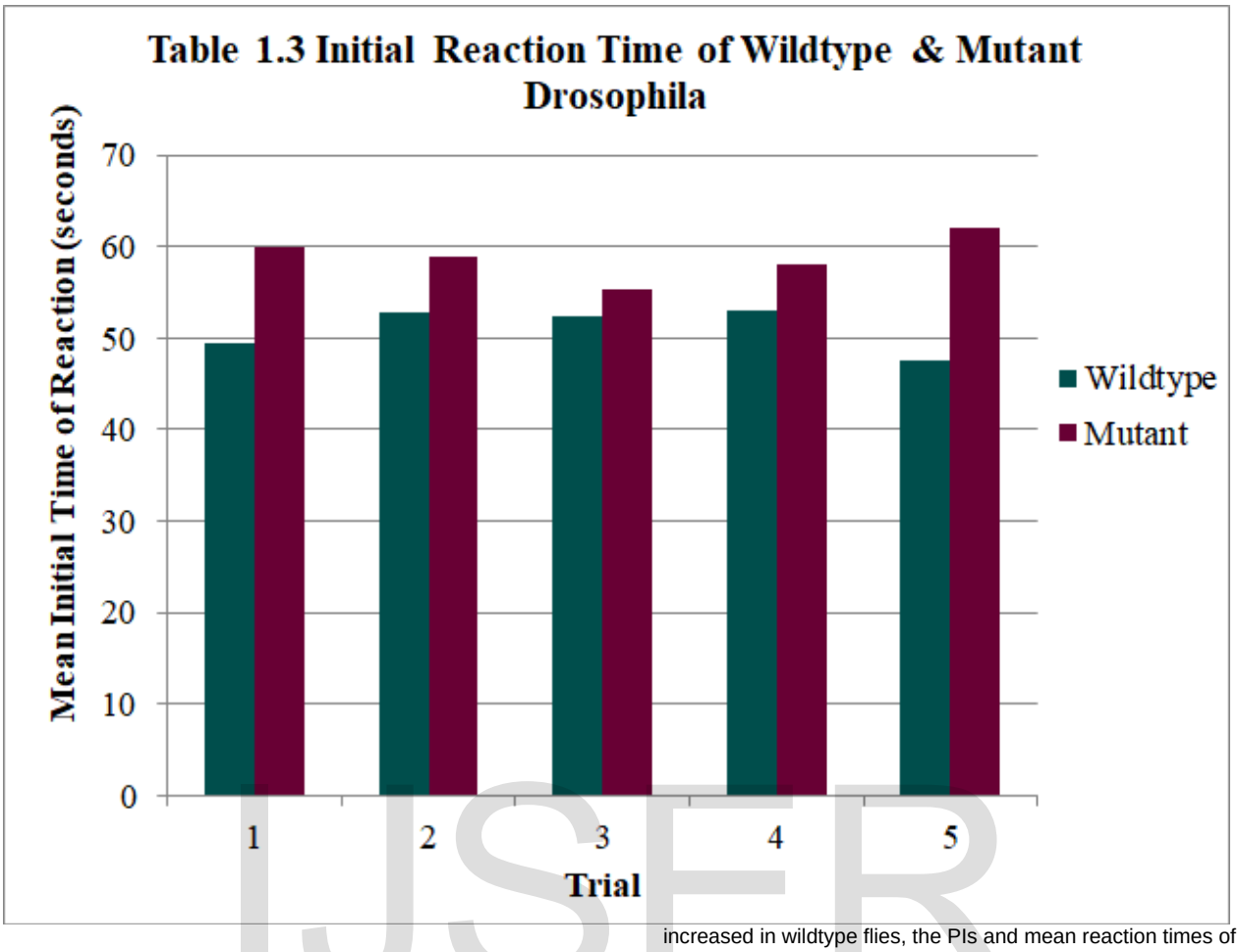
Illustrations

Graph 1.1 Mean Percent Change In Reaction Time of Mutant and Wildtype Drosophila



Graph 1.2 Mean Change In Performance Indices of Mutant and Wildtype Drosophila





Discussion

By the data, we reject the null hypothesis and support the alternative hypothesis, as there is a significant difference in the original mean reaction times of wildtype and mutant Drosophila.

Data suggests that the olfactory system of the mutant Antennapedia *Drosophilamelanogaster* is inferior to that of the wildtype *Drosophila melanogaster*. The mean reaction times of wildtype and mutant *Drosophila* were compared in a t-test for each trial. Four of the five t-tests yielded p-values less than 0.05, therefore suggesting a significant difference in the reaction times of the wildtype flies and mutant flies. By comparing the mean reaction times of the wildtype and mutant groups prior to treatment, it can be determined that the mean reaction times of the wildtype groups were less than those of the mutant groups. The wildtype group mean reaction times were 49.4 ± 10.9 , 52.7 ± 5.8, 52.4 ± 9.7, 53.1 ± 6.9, and 47.6 ± 6.2 seconds for trials 1 through 5, respectively. The mutant group mean reaction times were 60.0 ±9.6, 58.8 ± 9.7, 55.4 ± 6.9, 58.0 ± 7.3, and 62.0 ± 8.4 seconds for trials 1 through 5 respectively. As each mean reaction time for the wildtype flies was lower than the mean reaction time for the mutant flies in the corresponding trial, it was concluded that the wildtype flies have better-developed olfactory senses than the mutant flies. This can also be concluded as the overall percentage decrease in mean reaction time of wildtype flies was greater than those of the mutant flies, as shown by graph 1.1. Additionally, as indicated by figure 1.2, the change in PI of the wildtype flies was higher than that of the mutant flies for all five trials, which supports the conclusion that the wildtype flies exhibit better-developed olfactory systems because increases in PI indicate that more flies detected the scents used in the olfactory avoidance experiment.

Although the mutant flies were shown to have less-developed olfactory systems, the reaction times and performance indices after treatment was applied was nonetheless affected as shown infigures 1.1 and 1.2. Whereas a majority of the reaction times decreased after treatment was applied and the performance indices increased in wildtype flies, the PIs and mean reaction times of about half the mutant flies stayed nearly the same and the other half exhibited similar results as the wildtype flies, but to a lesser extent. Although the change in these values was less drastic than that of the wildtype groups, the treatments affecting approximately half of the mutant flies suggests that the mutant flies have an olfactory senses, despite them being less-developed than those of the wildtype flies. Treatments such as the alpha-lipoic acid and coffee seemed to have the most significant effect on the mutant flies as groups treated with coffee and alpha-lipoic acid exhibited the greatest percentage change in mean reaction times. However, exceptions include trial 4 in which the group treated with the garlic exhibited a 10.5% decrease in mean reaction time whereas the group treated with coffee exhibited a 6.6% decrease. Similarly, in trial 5 the group treated with combination of garlic and coffee exhibited a 13.5% decrease in mean reaction time whereas the group treated with the coffee exhibited a 10.7% decrease in mean reaction time.

In concern with the effect of each treatment, the null hypothesis was rejected and the alternative hypothesis was supported, as there was a significant difference in the mean reaction times before and after treatment in mutant and wildtype Drosophila. This was with the exception of the garlic treatment group in trials 3 and 5. Further, the null hypothesis was rejected and the alternative hypothesis was supported, as there was a significant difference in the mean reaction times of wildtype Drosophila groups with no treatment and treatment.Three out of five trials showed a significant difference between the combined treatment and control, garlic treatment and control, and the coffee treatment and control. All five trials showed a significant difference between the alpha-lipoic acid and control. As the majority of t-tests for each treatment conveyed the treatment as being significantly different from the control, it is suggested that each treatment did have a profound impact on the fly olfactory systems. In each of the trials, the treatment had a substantial effect on the reaction times of the wildtype *Drosophila* as the overall mean reaction time decreased by 36%, 48.6%, 23.7%, 26.4%, and 18.3% for trials 1 through 5 respectively. As for the performance indices, treatment

improved the overall average performance indices by 0.4, 0.3 0.5, 0.3, and 0.3 for trials 1 through 5 respectively. Due to the decreasing reaction times and increasing performance indices of the fly groups after treatment was added, as well as the significant differences between the treatments and control, it can be concluded that the treatments all had a positive effect on the olfactory systems of the wildtype *Drosophila*.

When comparing treatments against one another, the null hypothesis was supported and alternative hypothesis was rejected as there was no significant difference in the mean reaction times of wildtype Drosophila group with different treatments.When comparing each individual treatment to one another, t-tests for each trial conveyed that there is no significant difference between any of the treatments. All t-tests yielded a p-value above 0.05 between coffee and alpha-lipoic acid, combined and garlic, and garlic and coffee. At least four of five trialsshowed no significant difference between each treatment group. As the majority of treatments were shown as being significantly different from the control group but not significantly different from one another, it was concluded that all treatments were effective and, although some treatments may have been more effective than others, it was not enough to be seen as significantly different from the other treatments. By analyzing the graphs and data, we can determine which of the treatments were more effective than others. The alpha-lipoic acid and coffee are the most effective treatments as they had the greatest negative effect on reaction time and positive effect on the performance indices of the flies. The exceptions of this are the garlic in trial 1 and the combined treatment in trial 3, as they both resulted in a greater percentage decrease in reaction times in wildtype flies. Another exception was the combined treatment in trials 4 and 5, as it had an equal effect on the increase in the performance index as coffee and alpha-lipoic acid in each trial, respectively.

Conclusion

The data supports the conclusion that treatments do have an effect on the olfactory systems of *Drosophila melanogaster*. Although the mean reaction times and performance indices of the chemical treatment seemed to be more effective, the t-tests indicated that there is no significant difference between the natural and chemical treatments. If one were to recommend a natural treatment based off of these results, it would be likely be coffee; however garlic and the combination treatment are effective as well. Therefore, any of these treatments can be applied to insects in order to improve their olfactory systems--note that this will have a direct impact on the agriculture industry as insects serve as a basis for the environment. As the research additionally sought to compare the olfactory systems of mutant and wildtype *Drosophila*, it can also be concluded that the mutant *Drosophila* have less-developed olfactory systems than wildtype *Drosophila*.

During the experiment, errors that may have occurred include how much lemon juice and apple cider vinegar was added to the cotton pads during the olfactory avoidance experiment. Oftentimes, a small portion of liquid was caught and remained in the disposable pipet. Due to this, there may have been more or less liquid added to the cotton pad for each fly group; however, the extra or missing amount of liquid was likely minimal and trivial. Human reaction time must also be considered, as the reaction times of each fly was recorded by observing and timing the length of time the fly took to travel from the center to the opposite side.

As the data supports the conclusion that, although the antenna is the primary organ necessary for olfaction, there are other olfactory organs because the mutant flies did not have antennae and at least half of them were still able to detect scents, as shown by the increase in PI and decrease in mean reaction time after the treatment was added. As the maxillary palps are also known as olfactory organs in *Drosophila*, future studies may involve using Antennapedia mutant Drosophila melanogaster that do not have maxillary palps. By performing the same experiment on this mutant, the performance indices and reaction times can be studied in order to conclude whether other organs besides the maxillary palps and antennae of the fruit fly are involved in detecting odors. This would be suggested if the mutant displays an increase in PI and decrease in mean reaction time after the treatment is added as this would suggest that the flies were able to detect odors despite their lack of antenna and maxillary palps. As the number of treatments that could be used in the experiment were limited due to time and sample size, future studies could also use additional natural and chemical treatments. Castor oil, ginger, and cloves can be used as natural treatments, and chemical treatments may include current anosmia treatments in humans such as Pentoxifylline and Theophylline. More trials can be performed with a greater sample size of fruit flies in order to decrease variability.

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