The Journal of Experimental Secondary Science

The Effect of Red Light Stimulus on the Foraging Behavior of *Drosophila melanogaster* Through Measuring the Proboscis Activity

Hokyung Jennie Keum[1]*, Don Gibbs[2], and Bill Martin[2]
Student[1], Teacher[2]: Pomfret School, 398 Pomfret Street, Pomfret, CT 06258
*Correspondence: keumh.12@pomfretschool.org

Abstract

***Drosophila melanogaster* is commonly used as a model organism for biological research. A behavioral study on the effect of red light on fruit flies was conducted, asking specifically if a positive stimulus, red light, has an amplifying effect on *D. melanogaster's* craving behaviors. Another question asked in this study was if red light alone can train these organisms to anticipate food and to adopt specific foraging behaviors, even when food is not present. The proposed hypothesis was that when experimental *D. melanogaster* are conditioned with red light stimulus, the average amplitude of their foraging response would be greater than the average amplitude of the response recorded in control flies when they were presented food. Such a result would indicate that red light has a positive effect on these flies' response to food. According to the two sample T-test, the mean amplitude in the test conducted after the fifth trial in both the control and experimental groups were statistically equal (t-value=0.5169). However, the experimental group had statistically greater net average amplitude of response to food (t-value=-3.50), and the experimental group had statistically faster average response time (t-value=2.037). The data supported the hypothesized positive effect of red light on the flies' foraging behaviors. The data also suggested that the red light alone could not train the flies to successfully associate red light to food.**

Introduction

Many of the behavioral studies of *Drosophila melanogaster* conducted to date have involved a negative stimulus and a positive outcome. Mutiple studies that have questioned the ability of *D. melanogaster* to associate a certain stimulus to the anticipated behavior have been performed to date. In Mery's experiment[1], a repugnant chemical was used as a conditioning stimulus. The negative chemical stimulus was used to train the flies to avoid a certain fruit as an ovipositing site[1]. As a result, a significant number of experimental flies developed a connection between the repugnant chemical stimulus and the correct ovipositing sites, achieving success faster than the control flies.

Unlike Mery, Chabaud used a positive olfactory stimulus to train *D. melanogaster*[2]. This study examined a fly's proboscis, an elongated appendage that is positioned on the flies' head, and the flies were used for feeding. Response to the introduction of banana odor was rewarded with sucrose. Using the positive banana odor as the conditioning stimulus, *D. melanogaster* were trained to exhibit a strong activity in the proboscis, anticipating a food reward[2].

Dobzhansky's experiment on phototaxis and geotaxis in *D. melanogaster* offered evidence that *D. melanogaster* responds photopositively to red light[3], thus making it a positive stimulus. Under red light, which had an intensity of 0.2 lux, a photopositive population of flies remained photopositive and the photonegative population became either photopositive or photoneutral[3], suggesting that to *D. melanogaster*, red light is a positive stimulus.

Dobzhansky's experiment[3] gave an interesting result that suggested the possible usage of red light as a positive stimulus. The experimental results from Mery and Chabaud[2] suggest that *D. melanogaster* is capable of associating one stimulus to an anticipated behavior such as the extension of the proboscis. Combining the conclusions from the three experiments from Mery, Chabaud and Dobzhansky, the central question of this research became whether a positive stimulus (red light) can magnify *D. melanogaster's* behavior toward food and whether *D. melanogaster* can demonstrate a connection between the positive stimulus and a positive outcome. The methodologies applied here to investigate the effect of red light on the foraging behavior in fruit flies follow closely the techniques employed in the three works cited above.

In this study, 18 male vestigial-wing *D. melanogaster,* conditioned under red light from the time they were in the pre-pupa stage, were collected to serve as the 'experimental group'. The experimental flies were starved for six hours before experimentation. For five trials, *D. melanogaster* were presented with 20 seconds of red light, during which they were also presented with a Q-tip soaked with 0.01 M sucrose. After the fifth trial, a test of *D. melanogaster's* ability to associate red light with food was conducted - the test involved showing the fly just red light, and measuring its proboscis activity, a foraging behavior. There was also a control group, which again came from various single generations of male vestigial-wing *D. melanogaster*, and the control flies were also starved for six hours before the experimentation. The control group was presented with a Q-tip soaked with 0.01 M sucrose to the proboscis for five trials, but not red light. After the fifth trial, the feeding response of the control flies was tested by presenting the animals with a clean Q-tip; this same test was conducted for the experimental population, with the Q-tip accompanied by red light. For both groups, the amplitude of response was measured using the 6-point system.

Materials and Methods

Control Group:

A total of 18 male vestigial-wing *D. melanogaster* were used. After anesthetizing the flies with FlyNap® (Carolina Biological Supply Company), male flies were separated from females. Only two flies were tested at a time. Selected male flies were mounted onto a paraffin wax-wrapped toothpick secured at the base of the wings. By poking a small hole (the width of toothpick) on the polyurethane foam vial cap, the fly-attached toothpick was securely placed into the hole made on the foam cap (Figure 1).

The flies were then kept in the vials for a six-hour starvation period. After 6-hours, the fly, still attached to the toothpick, was placed under the dissecting microscope, proboscis side up. Before beginning each trial, the amplitude of spontaneous movement of the proboscis (initial state of the proboscis) was measured. Then, the amplitude of response to access to food, including the time it took for that specific response, was measured. The amplitude (amplitude of response to access to food – spontaneous response) was measured through the 6-point system (Figure 2). The spontaneous movement of the proboscis was the amplitude of the control response exhibited by the fly when a clean, dry Q-tip was introduced. The Q-tip was placed where the fly's proboscis almost touched the head of the Q-tip. Then, the amplitude of the response to food was judged when a Q-tip soaked with 0.01M sucrose was made available. The flies were given a maximum of 20 seconds to exhibit a response to the sucrose Q-tip. If the fly still did not show any response after 20 seconds, the response time was still recorded as 20 seconds. Sucrose was given as a reward after a positive response. This two-part trial was repeated five times with 5 minute resting intervals between each trial. Continuously testing three flies at a time inevitably caused each fly to have a waiting period. Unless a uniform interval of waiting period was assigned, the waiting period of each fly would be irregular. By setting the waiting period (and calling it interval of resting) to be five minutes, each fly had equal interval of waiting in between each trial, and fives minutes was the minimum amount of time needed to finish one trial without any rush. Right after the fifth trial, testing for learned behavior was conducted: first, the natural position (movement) of the proboscis was recorded. Then, a clean Q-tip was introduced near the fly's proboscis, and the amplitude of response was recorded. The purpose of this test was to see if the control flies had associated the Q-tip with food.

Experimental Group: Eighteen vestigial-wing male *D.melanogaster* grown under red light since birth were used as the experimental group. Two flies were trained at a time. The flies were mounted on the paraffin waxed-wrapped toothpicks using the same procedures and equipment used to prepare the control group. These experimental flies were also kept in vials containing moist cotton balls. The only difference was that next to the hole on the polyurethane foam, there was another hole to hold the red light. Therefore, experimental flies were constantly exposed to red light from the time of birth to the time of experiment in order to increase the flies' familiarity with the red light. The actual experiment began after 6-hours of starvation time. The dissecting microscope light was not turned on; only the natural lighting coming in through windows was present. Each trial began with measuring the spontaneous response—a clean Q-tip was introduced near the fly's proboscis area. With red light in hand, the fly, under the dissecting microscope, was exposed to red light for 10 seconds. After 10 seconds, a sucrose-soaked Q-tip was introduced to the fly (with red light still shining down on the fly simultaneously). The fly was exposed to red light longer than sucrose soaked Q-tip. The fly grew since the pre-pupa stage feeding in the food vial under the red light. This pre-conditioning the fly received led to the anticipation that 10 seconds of exposure to red light prior to 10 seconds of access to food will aid the fly to better express foraging behavior. If the fly showed response, sucrose was given to the fly as a reward. A total

Figure 1. Vial containing a water soaked cotton ball and a polyurethane foam cap holding a toothpick attached with a test subject fly. Each of the two flies was kept in separate vials equipped with a moistened cotton ball to prevent the flies from dehydrating. The toothpick was inserted close enough to the moistened cotton ball for the fly to extend its proboscis for water.

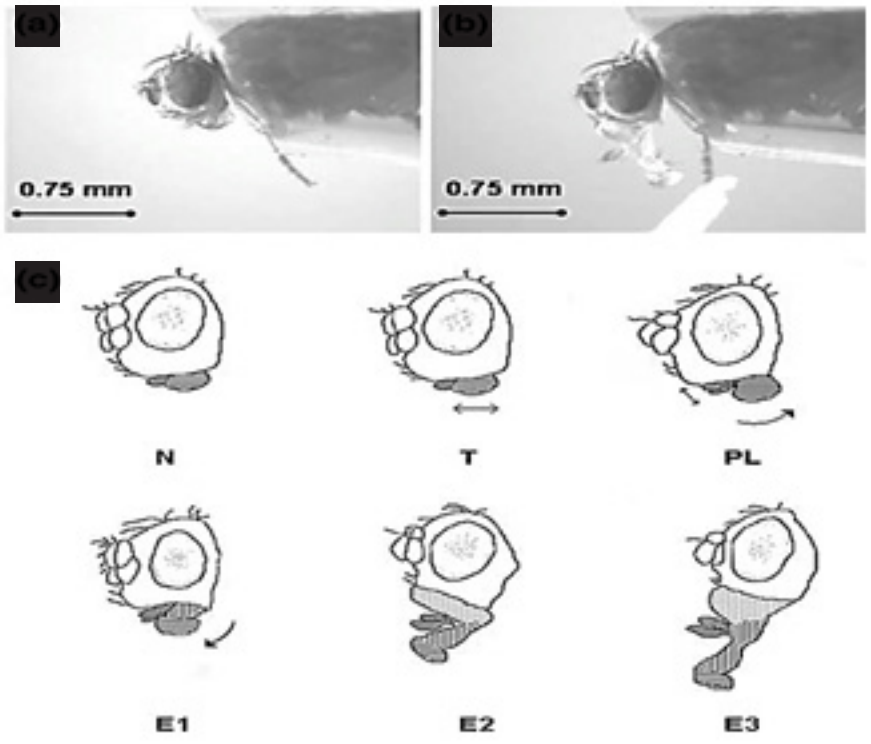

Figure 2. The 6-point System used to measure the amplitude of response. Maxillary palps (grey), Labellum (horizontal hatchings), Haustellum (vertical hatchings), Rostrum (dots).
N (0 point): no movement, T (1 point): trembling of the labellum, PL (2 points): trembling of maxillary palps and/or slight extension of the labellum, E1 (3 points): slight extension of the haustellum, E2 (4 points): extension of the haustellum and partial extension of the rostrum, E3 (5 points): full extension of the proboscis
**Adapted from Chaubaud, et al. 2006*

The Journal of Experimental Secondary Science

of five trials were carried out, and there was a five-minute resting period between trials. Immediately after the fifth trial, a learning test was conducted - the fly's natural position of the proboscis, without the introduction of a clean Q-tip, was recorded. The fly was then exposed to red light for twenty seconds, or less if the fly showed a response. The purpose of this learning test in the experimental group was to see if the flies had associated the red light stimulus with food. The data collected from both the control and experimental group were analyzed through series of statistic tests: two sample t-test with σ=0.05. A total of four different, two sample t-tests were carried out (Table 1).

Results

The results from table 2 describe the control group flies' response when they were exposed to the clean Q-tip and food, and when they were tested for learned behavior. The results from table 3 describe the experimental flies' response when they were exposed to the clean Q-tip and food given while shining red light, and when they were tested for learned behavior.

In order to analyze the data, two sample T-test (σ=0.05) was used. A total of four statistic tests were conducted to verify that Q-tip is an ignorable variable in the experiment, the experimental group flies show significantly stronger response to the food source, the experimental group flies show significantly faster response to the food source, and the learning test response in both groups are statistically equal (Table 4).

In both the control and the experimental group, average amplitude of response to the clean Q-tip showed little variance and only a few data points showed significant deviation from the mean (meancontrol=0.5722, meanexperimental=0.4).

Because the data of the average amplitude of response to food in the control group compared to that of the experimental group's seemed to differ significantly, a graph was created as a visual aid to seeing the data difference (Figure 3).

Table 1. Hypothesis for four different two sample t-tests.

	H_0	H_a
Test 1	The mean initial response in control flies is equal to the mean of initial response in experimental flies.	The mean initial response in control flies is not equal to the mean of initial response in experimental flies.
Test 2	The mean amplitude of the feeding response seen in control flies is equal to the mean of amplitude of response in experimental flies.	The mean amplitude of the feeding response seen in control flies is less than the mean of amplitude of response in experimental flies.
Test 3	The mean response time in control flies is equal to the mean of response time in experimental flies.	The mean response time in control flies is greater than the mean of response time in experimental flies.
Test 4	The mean amplitude of the response to the learning test in control flies is equal to the mean of that response in experimental flies.	The mean amplitude of the response to the learning test in control flies not equal to the mean of that response in experimental flies.

Table 2. Average data of the control group flies conditioned with red light stimulus.

Fly#	Avg. Amplitude of Spontaneous Response to Clean Q-tip (N=5)	Avg. Amplitude of Response to Access to Food (N=5)	Avg. Response Time (N=5)	Amplitude of Learning Test Response
1	0.4±0.489	1±1.549	15.4±5.713	1
2	0	0.2±0.4	20	0
3	2.6±1.2	2.2±1.166	1.8±0.4	2
4	1.2±1.469	4.4±1.469	7.4±3.322	2
5	3.2±1.095	1.4±2.227	14±4.898	1
6	0.4±0.748	2.8±2.315	13.4±8.236	-1
7	0.8±0.4	1±1.549	12±7.042	0
8	0.4±0.489	0.8±1.720	15.4±6.053	0
9	0	5	6.4±5.986	0
10	0.8±0.4	4.2±1.6	3.4±1.019	0
11	0.5±0.489	0.4±1.356	18±4	0
12	0	4.2±1.6	6.6±7.337	1
13	0	4.6±0.489	6.6±7.337	0
14	0	4.8±0.4	2±4.127	0
15	0	5	1.4±1.095	5
16	0	5	1.8±0.4	0
17	0	1.8±1.939	11.4±1.166	0
18	0	2±2.449	13.6±7.380	0

The values are the average of five trials of spontaneous response, response to access to food and response time. Learning test is not averaged since only 1 trial of the testing was done for each fly. In the case of a -1 response amplitude, initial response (natural position of the proboscis before testing) was greater than the response to the learning test.

Table 3. Average data of the experimental group flies conditioned with red light stimulus.

Fly#	Avg. Amplitude of Spontaneous Response to Clean Q-tip (N=5)	Avg. Amplitude of Response During Red Light + Food Conditioning (N=5)	Avg. Response Time (N=5)	Amplitude of Learning Test Response
1	1.8±2.227	3.2±2.227	5±2	5
2	0	4.6±0.8	12.6±5.388	0
3	0.8±1.6	4.2±1.6	4.8±2.925	0
4	0.2±0.4	4.6±0.8	6.6±4.498	0
5	0.6±1.2	3.4±1.2	7.2±2.059	0
6	0.6±1.2	4.4±0.979	4±2	0
7	0.2±0.4	4.8±0.4	4.2±4.498	0
8	0.4±0.489	4.4±0.979	5.4±2.576	1
9	0	4.4±0.979	6±2.828	0
10	0.8±1.6	4.2±1.6	5.2±2.925	1
11	0	5	9±2.607	0
12	0.4±	4.4±0.979	8.6±2.727	0
13	0.4±	4.6±0.8	3.6±0.8	1
14	0.4±	4.2±1.6	6.2±2.561	0
15	0	4.8±0.4	6±2.828	0
16	0.2±	4.2±1.6	5.8±2.135	0
17	0.4±	4.4±0.979	6.4±3.878	0
18	0	4.6±0.8	8.4±3.072	-1

The Journal of Experimental Secondary Science

Discussion

The result of statistical test 1(Table 4), on the spontaneous response in both control and experimental groups, failed to reject H_0_t=0.7133. This indicated that the average spontaneous response in control and experimental flies are equal, or there was no statistically significant difference. The clean Q-tip did not have a significant role in one group over the other. Therefore, Q-tip was not an interfering variable in the experiment—any response to sucrose-soaked Q-tip can be attributed to the food and not to the instrument.

Table 4.Two sample t-test results

Test (α=0.05)	$Mean_{control}$	$Mean_{experimental}$	T-value	P-value	Result
1	0.5722	0.4	0.7133	0.4824	Failed to reject H_0
2	2.822	4.355	3.504	0.00118	Rejected H_0
3	9.478	6.389	2.037	0.0271	Rejected H_0
4	0.6111	0.3889	0.5169	0.6068	Failed to reject H_0

The result of statistical test 2, on the average amplitude of response during training, rejected H_0_t=3.504. This showed that the experimental group exhibited a greater overall response to food (sucrose). This suggests the positive effect of red light on the flies' response to food—the feeding response is magnified when flies are conditioned with red light. By being conditioned with red light continuously from the pre-pupa stage and through the five trials, the experimental flies may have become familiar with the red light. This familiarity in turn could have stimulated the taste sensors of these flies. The control group did have exhibit response to food, but not as strong and consistent as the experimental group.

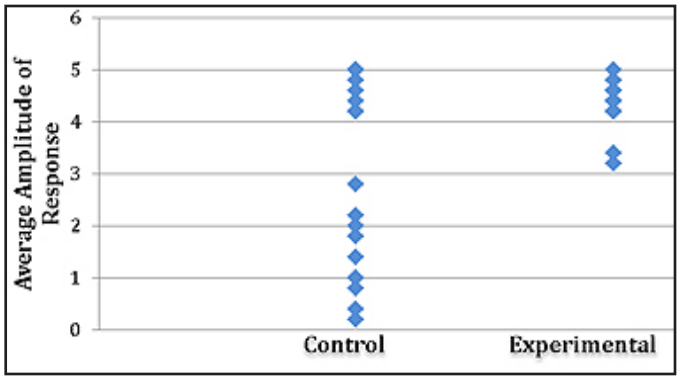

Figure 3. This graph is a comparison between the control and experimental groups on the average amplitude of response to food. (N=18; 5 control group data points and 11 experimental group data points appear as if they are not represented in the above graph because these data points overlap with other data points that have the same amplitude of response).

The result of statistical test 3 conducted on the average response time in both groups rejected the H_0_t=2.037. Thus, the control group exhibited a slower response than the experimental group. Again, this suggests that red light had a positive effect on the experimental flies' response to food: the flies responded to food more quickly when conditioned with red light. The first 10 seconds of exposure to red light without the introduction of sucrose-soaked Q-tip could have served as an informing stimulus, hence accelerating the experimental flies' response to food. The positive of effect of red light on the flies' response to food is easier to see in Figure 3. The graph shows that the majority of the experimental flies have exhibited average response amplitude of above 3. However, for the control group, the majority of the flies exhibited average response amplitude of below 3.

Combining the results of all three statistical tests conducted on the data from table 4 and figure 3, the hypothesis, stating that the experimental group's overall amplitude of response will be greater than that of the control group's, was supported by the data of the experiment. By conditioning the flies with red light, the flies showed overall a greater and timelier response to food. Dobzhansky's experiment concluding that *D. melanogaster* favors red light is supported in this experiment: red light not only increased the intensity of the flies' response to food, but it also quickened the flies' response to food3. Considering these two conclusions that has been made from the data, conditioning flies with red light effects the flies perhaps mentally. Red light alone (referring to table 2 and 3 'amplitude of response to learning test') does not trigger any significant response (table 4, statistical test 4 - t=0.5169), but red light with sucrose amplifies the proboscis activity. This leads to a possibility that the olfactory neurons were stimulated by the red light to amplify the flies' ability to detect the sweet odor of sucrose and thus amplifying the activity of the flies' proboscis and accelerating the flies' response to food upon the introduction of sucrose.However, whether or not red light directly increased the flies' foraging behavior by stimulating the taste sensors is unknown. The effectiveness of red light as a learning device for *D.melanogaster* is hard to confirm. Statistical test 4, on the average response during the learning test in both groups, showed that there was no significant difference between the two groups' proboscis activity. Therefore, red light was not associated with food nor did it trigger an anticipatory response in the flies, even though significantly larger behavioral responses to food were seen in the experimental group. Thus, it would not be safe to conclude that red light successfully conditioned the flies to anticipate food. Instead, according to statistical test 2 and 3, red light did have an effect on how strongly and how fast the flies responded to access to food.

Certainly, there were some errors, which could have been fixed. First, more than five trials should have been conducted for each test subject. Increasing the number of trials could have improved the results of the learning test, possibly altering the conclusion that red light is not an effective learning device. Five trials of conditioning resulted in the amplification of the foraging behavior and the quickening of the flies' response to food. More trials could have resulted in the flies actually associating the red light stimulus to sucrose. Also, using the Q-tip to feed the flies may have been a poor choice because the cotton head was far larger than the flies' very small proboscis. Sometimes the sucrose solution from the Q-tip covered the whole head of the flies, hampering the collection of quality data. Instead, a micropipette could have been used to feed the flies. The sucrose solution would not easily drop to soak the flies with sucrose because the solution is

kept in the pipette unless it is squeezed out. The flies can feed on the solution by sinking its proboscis through the small opening of the pipette where the sucrose solution is suspended because of the solution's surface tension.

Another error that should have been thought out was exposing red light to the experimental group prior to the experimentation. The initial logic was that early exposure to red light was what distinguished the experimental group from the control group. Early exposure to red light had been incorrectly used as a part of the conditioning process of the experimental group for conditioning the experimental flies with red light from the pre-pupa stage created another variable. The increased magnitude of foraging behavior in the experimental group could have been affected by this extra variable.

An inconvenient choice was the use of vestigial-wing flies. Vestigial flies have very small, deteriorated wings, which made mounting these flies onto toothpicks difficult and time consuming. Many times the flies detached itself from the toothpick because it was easy for the flies' small vestigial wings to rip off from either the toothpick or the flies' bodies. These flies walked off from the microscope and test subjects were many times lost as a result. As a result, data was ruined, and the ruined data had to be discarded.

Another difficulty was running the risk of hurting the flies with the heated dissecting needle. Because the dissecting needle was not thin enough, sometimes the needle touched the bodies of the flies instead of its wings. Whenever the hot needle touched parts of the flies other than the vestigial wings, the flies' abdomens curled up and proboscis extended immediately. These behaviors seemed to be signs of pain. Flies that have been burned by the hot dissecting needle were found to die easily, not surviving long enough to conduct the five trials of experiment; this error served to slow down the data collecting process. Using wild type flies would have been more ideal since their wings are large, giving more surface area of attachment onto the toothpick.

In the next experiment, many of these errors will be corrected, and the reason behind why red light amplified and quickened the foraging behavior of the flies will be studied. Also, this research will be redesigned to focus on the effectiveness of red light as a learning device. The ability of the flies' to associate one stimulus to another will be one of the central questions in hand.

References

1. Mery, F., Kawecki, T.J. (2002). *Experimental Evolution of Learning Ability in Fruit Flies.* Proceedings of the National Academy of Sciences, Vol.99 No.22, 14274-14279.

2. Chabaud, M., Devaud, J., Pham-Delègue, M., Preat, T., and Kaiser, L. (2006).*Olfactory Conditioning of Proboscis Activity in Drosophila melanogaster.* Journal of Comparative Physiology, Vol.192 No.12, 1345-1388.

3. Dobzhansky, T., Judson, C.L., Pavlovsky, O. (1974), *Behavior in Different Environments of Populations of Drosophila pseudoobscura Selected for Phototaxis and Geotaxis.* Proceedings of the National Academy of Sciences, Vol.71 No.5, 1974-1976.

Acknowledgements

Thank you Dr. Chun Geun Lee, MD, PhD, MSc at Yale University for always sparing some time to answer my conceptual question on fruit fly research.