Observing Locomotor Behavioral Responses with the Optogenetic Stimulation of Drosophila at Varying Light Source Distances

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Abstract

Understanding how optogenetic stimulation works opens more research possibilities for neuroscientists when it comes to being able to activate specific neurons with higher precision and efficiency. Optogenetics is also part of a group of other genetically-controlled neural manipulation types, and the fruit fly optogenetic model can serve as an introduction to this field. With the fruit fly model, we decided to test varying distances of light stimuli on the locomotor responses of the optogenetic flies. Using a red-orange (617 nm) LED light to stimulate the flies and a ruler to measure length, we recorded the proportion of exhibited locomotor behaviors at different distances of light. We observed that the Type 2 flies exhibited behavior that resembled optogenetic activation of motor neurons. It was also observed that approximately 10 cm served as a threshold for the optogenetic flies to exhibit motor behavior 100% of the time for a 1 second light stimulus. These findings help us understand the sensitivity of the optogenetic channels and what the neurons are capable of controlling. As a result, we can learn the limitations of this technology and apply this knowledge when performing other experiments involving optogenetics.

Methods

Fly Preparation

Three flies from each of the two types were obtained and were each assigned a number 1 to 3. Each fly was put into a petri dish which served as a holding chamber to administer the red-light stimulus and to observe their resulting responses. The petri dishes were labeled in permanent marker with the fly's assigned number and whether they were Type 1 or Type 2 flies.

Preparation of Recording Setup

The following step explained in detail was performed in order to collect data on how light intensity is affected by the distance of light. The procedure was done to gather additional insight and is not directly related to the purpose of the investigation. The phone app Lux Light Meter Free was used to record the intensity of light in lux, or lumens/m^2.

A ruler was used for all steps involving measuring length to ensure that the distances are accurate. The distance measurements are defined as the highest point of the flashlight's notch (Figure 1) to the point where the light shines onto. In this case, the light shines onto the luxmeter. The flashlight would also be placed 0.4 cm higher on the ruler to account for the ruler's extra margin.

A petri dish lid was placed on top of the phone camera sensor to calibrate the luxmeter to match the light intensity the flies inside of the petri dishes would be exposed to. A red-orange light (617 nm) was fixed to shine 14 cm above the light intensity recording setup. The light intensity was recorded for every 2 cm decrease in distance until the light source was 2 cm above the luxmeter. The data is then displayed as a scatterplot using Excel. A quadratic fit was applied for the

trendline as 1 lux is defined as the lighting of an area of 1 m^2. The equation of the trendline and R^2 value was also calculated using Excel.

Experimental Procedure

First, data was collected starting with Fly 1 from the Type 1 group. The petri dish is placed on top of a blank white sheet of paper to allow for greater visibility of the fly. The red-orange LED light was shined 14 cm away from the fly for a duration of 1 second. While the red-light stimulus was given, the fly was observed for a locomotion behavioral response. A locomotion behavioral response was defined as twitching or frantic movement within the dish. If a locomotion behavioral response is exhibited, then a value of 1 would be recorded for that trial. A data value of 0 would be recorded if there was no locomotion behavioral response. This was repeated for a total of three trials and the fly was given a slight resting period of 10 seconds in between trials. These steps to record data were repeated after reducing the distance of the source of light to the bottom of the petri dish by 2 cm. Data was recorded at every 2 cm decrease until the flashlight was 2 cm away from the bottom of the petri dish. The protocol explained so far for the Fly 1 from the Type 1 group was repeated for the remaining five flies.

At each distance marker, there should be a total of 9 recorded data values for each type. The average of those 9 recorded data values were calculated to represent the proportion of flies exhibiting a locomotive behavior for a specific distance of light stimulation. These proportions for every level of distance and fly type were then plotted as a bar chart using Excel.

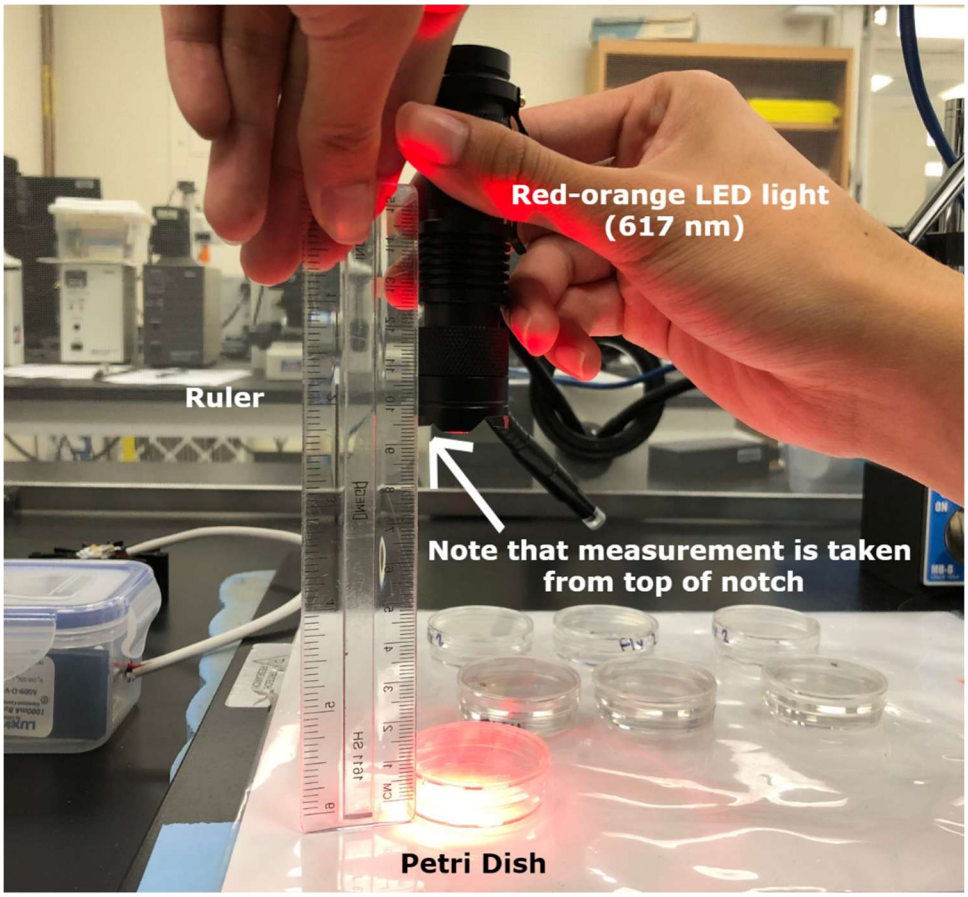

Figure 1. **Optogenetic fly red light stimulation setup for testing distance.** A labeled photograph that details the data collection protocol of the experiment. The metric side of the ruler is used to measure the distance in centimeters. An arrow points to the highest part of the notch of the LED flashlight, indicating where the distance measurement ends. The red-orange (617 nm) LED light is placed 0.4 cm higher than the distance value that is being measured for to account for the margin at the end of the ruler. The described protocol should approximate the distance from the source of light to the bottom of the petri dish.

Results

Table 1. **Raw data showing proportion of flies exhibiting locomotion behavioral response at varying distances of red-light stimulation.** There are 3 flies in the sample for each of the 2 types. For each fly, there are 3 trials of behavioral recordings at every distance marker. A value of 0 indicates no observed locomotion response, and a value of 1 indicates an observed response.

			Distance						
			2 cm	4 cm	6 cm	8cm	10 cm	12 cm	14 cm
Type 1 Flies	Fly 1	Trial 1	0	0	0	0	1	0	0
		Trial 2	0	0	0	0	0	0	1
		Trial 3	0	0	0	0	0	0	0
	Fly 2	Trial 1	0	0	1	0	0	0	0
		Trial 2	0	0	1	0	0	0	0
		Trial 3	0	0	0	0	0	1	0
	Fly 3	Trial 1	0	0	1	1	1	0	0
		Trial 2	0	0	0	0	0	0	0
		Trial 3	0	0	0	0	0	0	0
Proportion			0	0	0.33333	0.11111	0.22222	0.11111	0.11111
Type 2 Flies	Fly 1	Trial 1	1	1	1	1	1	0	0
		Trial 2	1	1	1	1	1	1	0
		Trial 3	1	1	1	1	1	1	0
	Fly 2	Trial 1	1	1	1	1	1	1	1
		Trial 2	1	1	1	1	1	1	1
		Trial 3	1	1	1	1	1	1	1
	Fly 3	Trial 1	1	1	1	1	1	1	1
		Trial 2	1	1	1	1	1	1	1
		Trial 3	1	1	1	1	1	1	1
Proportion			1	1	1	1	1	0.88889	0.66667

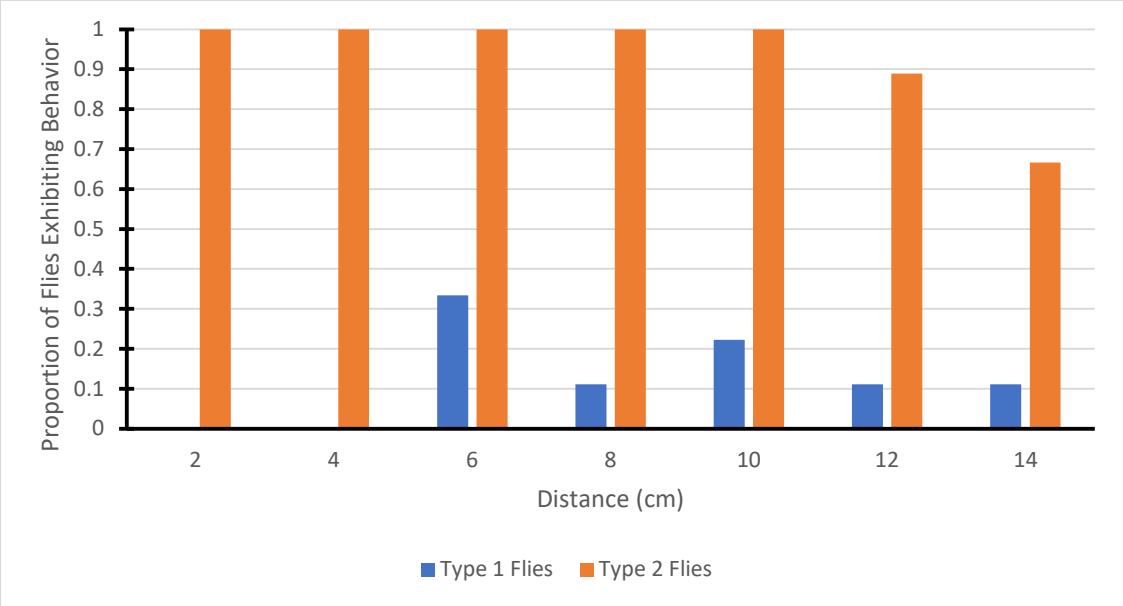

Figure 2. **Proportion of fruit flies exhibiting locomotion behavioral responses after administering red light stimulation at varying distances.** A bar chart comparing the proportion of flies exhibiting locomotion behavior between Type 1 (blue) and Type 2 (orange) flies at different distances of light stimulation. The units for distance of light are in centimeters. At each distance marker, there are 3 trials each for each of the 3 flies belonging to a group.

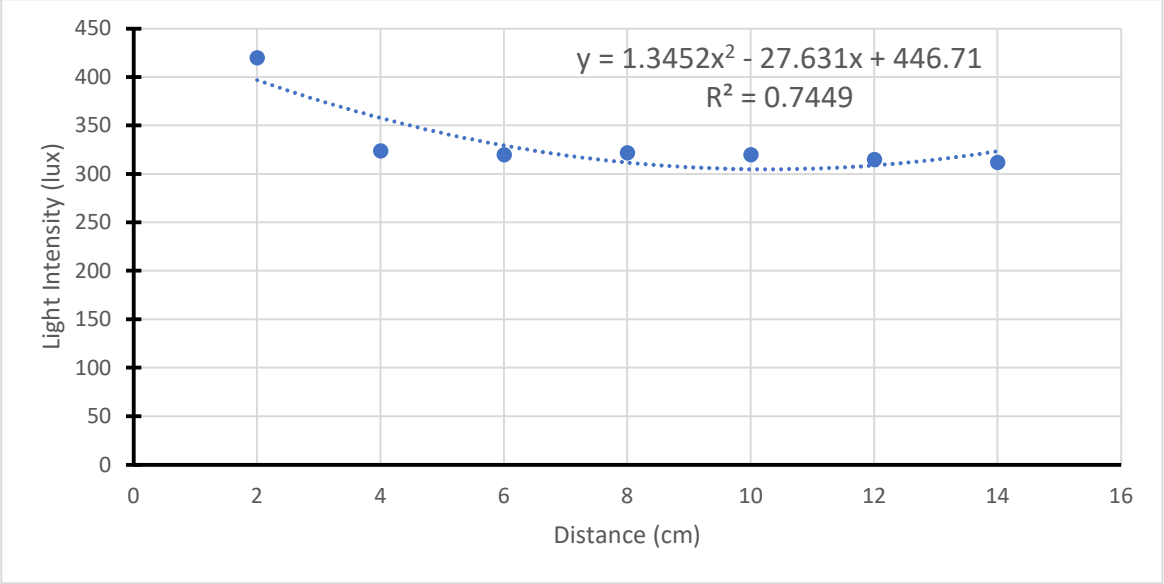

Figure 3. **Light intensity of a red-orange (617 nm) LED light with distance.** A scatterplot showing the light intensity of an LED light, measured in lux, recorded by the luxmeter phone app at specific distances away in centimeters. The fit for the trendline is exhibited as a second-degree polynomial function, or quadratic. The equation is $y = 1.3452x^2 - 27.631x + 446.71$ and the R^2 value is 0.7449.

While the variable being tested in this experiment is on the distance of light stimulation, further research on light intensity was done to gain further insight. The R^2 value from Figure 3 was found to be 0.7449 which shows a high level of correlation to the quadratic fit.

It was also observed that the Type 2 flies have a greater proportion of flies exhibiting locomotion responses compared to the Type 1 flies. For all trials within the Type 2 fly groups between 2 to 10 cm, the flies all showed a locomotion behavioral response. For all trials within the Type 1 fly groups between 2 to 4 cm, none of the flies showed a response.

Fly 2 and 3 from the Type 2 group exhibited locomotion responses for all trials. However, Fly 3's response to the light stimulus was observed to be more immediate and violent compared to Fly 2's behavior.

Discussion

In this experiment, we used optogenetic and wild type flies to further our understanding of how red light can be used to stimulate neurons. This experiment gives us insight into the possibilities of genetically-controlled neural manipulation so we can perform more various types of research now that we have easier access to turn off or on specific neurons. Analyzing the results, it was observed that the Type 2 flies had a greater proportion of flies exhibiting locomotion behavior compared to the Type 1 flies. This provides us with evidence that the Type 2 flies are the optogenetic flies while the Type 1 flies are the control wild type. It was also observed that around 10 cm may serve as a threshold to get the optogenetic flies to exhibit locomotion behavior 100% of the time. Although there is evidence supporting that Type 1 flies are not optogenetic, it was still observed that they exhibited some locomotive behavior on rare occasion.

It was observed in their behavior that some of the optogenetic flies were more sensitive to the stimulus compared to others within the same type. Fly 3 from the Type 2 group had the most intense reactions to the light and showcased more frantic movement. The health of the fly could be a factor to how much they respond. The behavior of this Fly 3 gave visible evidence that the optogenetic channels activates motor neurons to cause this movement response. Chrimson is used as it is a channelrhodopsin activated by red light.