# Visual perception of light intensity in *Drosophila melanogaster* wild type and *ort*$^1$ mutants

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**Abstract**

The objective for this study was to determine whether wild-type and *ort*$^1$ mutant *Drosophila melanogaster* differ in their perception of light intensities. Previous studies have shown that *Drosophila* exhibit greater movement towards brighter environments than dim places. We predicted that *ort*$^1$ mutants, which lack R1-R6 photoreceptors, would not perceive blue light to be as intense or bright as wild type and if there was no difference in their perception of light intensities they were expected to travel the same distance towards blue light. Due to red light colour-blindness in *Drosophila*, neither wild type nor *ort*$^1$ mutants were expected to move towards red light. We illuminated blue and red films on each side of a T-maze and measured the distance traveled by wild-type and mutant flies from the centre of the maze. Although a trend was observed indicating that wild type and mutants moved more towards blue light than red light, we found that wild type and ort$^1$ mutants did not differ significantly in the distance traveled towards blue light. Thus, we failed to reject our null hypothesis reflecting that there was no difference in the perceptions of light intensity between wild type and ort$^1$ mutants.

**Introduction**

The visual system of *Drosophila melanogaster*, the common fruit fly, has been studied to some extent over the years, particularly the pathways involved in light detection (Hardie 1985). However, relatively little research has been done on perception of light intensity in *Drosophila*, particularly in individuals with mutations in light detection pathways. In this study we hoped to learn more about how light intensity is sensed in *D. melanogaster* with respect to the photoreceptor cells of insect compound eyes. This knowledge may be applicable to other insects which possess compound eyes. The *D. melanogaster* compound eye is composed of 750 ommatidium units which contain two inner photoreceptor cells (R7 and R8) and six outer photoreceptor cells (R1-R6) (Hardie 1985). The inner photoreceptors contain rhodopsin pigments Rh3-Rh6 which detect narrow ranges of wavelengths of light for differentiating violet, blue and green (Yamaguchi *et al*. 2010). The outer photoreceptors contain the Rh1 pigment,

capable of detecting a broad range of wavelengths, as illustrated in Figure 1 (Yamaguchi *et al*. 2010). *D. melanogaster* has been found to be color blind for red, as their eyes lack a pigment capable of absorbing red light (Yamaguchi *et al*. 2010).

Mutations in the *ort* gene, which cause defects in histamine chloride channels (ort) of the eye result in the absence of R1-R6 outer photoreceptors. $Ort^{1}$ mutants lack R1-R6 outer photoreceptors but can still differentiate the same colours as wild-type *Drosophila* due to the presence R7 and R8 photoreceptors (O'Tousa *et al*. 1989, Gao *et al.* 2008).

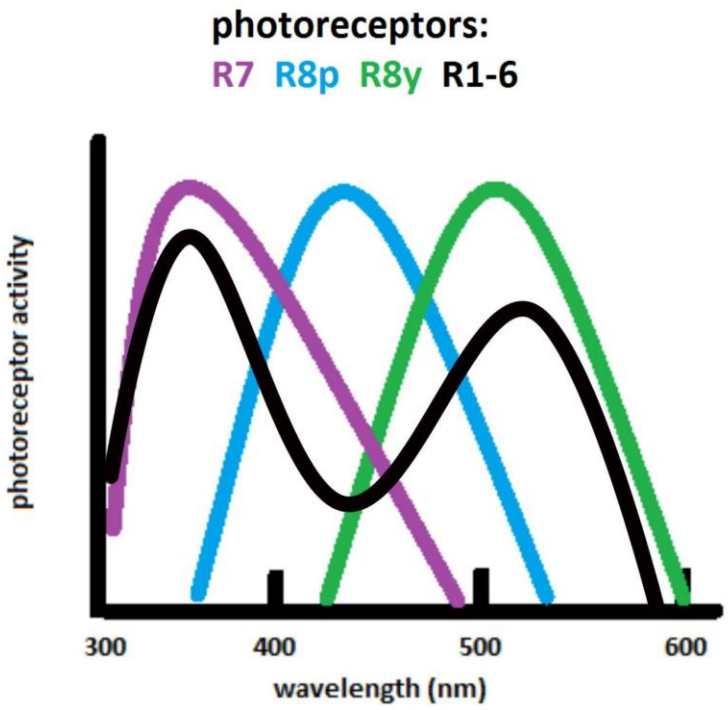


Figure 1. Wavelengths of light detected by photoreceptor cells with respect to photoreceptor activity in the eyes of *Drosophila melanogaster* (adapted from Yamaguchi *et al*. 2010).

As blue light should still be visible to both wild type and *ort*$^{1}$ mutant *D. melanogaster*, the objective of our experiment was to determine whether the *ort*$^{1}$ mutant perceives the intensity or brightness of blue light differently from wild type due to their lack of the outer photoreceptor cells. Keene *et al.* (2011) found that adult *D. melanogaster* exhibit greater movement towards brightly lit environments over dim places. Thus, our first alternate hypothesis was that blue light would not appear as bright to mutants, therefore mutants would not move as far towards blue light as would wild type. The null hypothesis was that wild type and *ort*$^{1}$ mutants would travel the same distance, or that mutants would travel farther than wild type towards blue light.

We also wanted to examine whether the *ort*$^{1}$ mutation would affect *D. melanogaster*'s red-light color blindness, as this has never been tested in *ort*$^{1}$ mutants, so the distance specimens traveled towards red light was measured as well. Unfortunately, the sample size of *D. melanogaster* that moved towards red was quite small (n=2 for wild type, n=5 for mutants) to perform a subsequent t-test. Instead, we looked for a general trend that wild type and *ort*$^{1}$ mutants would travel towards blue light more often than the red light, due to their red color-blindness and preference for brighter environments.

**Methods**

To begin our experiment we set up two lamps with 60 Watt light bulbs and placed them under a red film and a blue film respectively. We attached a 14 cm long glass T-maze to a clamp above the films and closed its ends with pieces of cotton. We measured light intensity from each lamp and adjusted their distance relative to the T-maze to ensure that they were giving off the same intensity (50 Lux). We turned off the room lights and performed the procedure with the lamps as the only light source in order to avoid extraneous light. We then removed a single fly

from a vial and inserted it into the T-maze entrance, as seen in Figure 2. The entrance was closed off with cotton in order to prevent the flies from moving backwards up the maze.

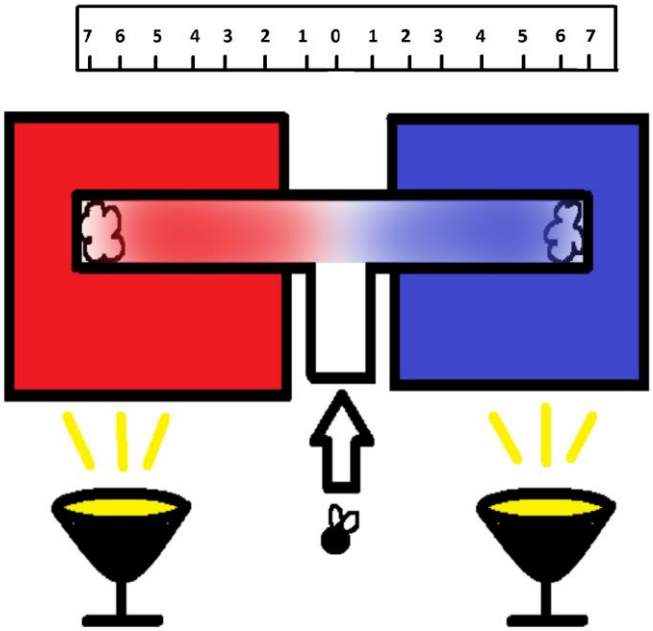


Figure 2. Experimental set-up for *D. melanogaster* color perception test. Lamps were used to illuminate red and blue film below a T-maze. Flies were released into the centre of the T-maze.

In order to remove a single fly from the vial at a time and to not harm the flies in the process, we devised a method for transporting them using a drinking straw. The straw was cut in half and inserted through the layer of cotton into the vial to the space with the flies. A single fly could easily pass through the straw and enter the T-maze with this method. As soon as a fly entered the T-maze, we blocked off the entrance with cotton and started a timer. We avoided forceps and other tools because they could clip the wings of the flies and injure them while being moved.

In order to measure the initial response to the light, we set a limit of 20 seconds and recorded the color that the fly chose after that time interval based on distance travelled. We

determined that twenty seconds was a reasonable time interval after experimental trials with the mutant and wild-type flies, prior to performing the actual experiment. During these experimental replicates we observed the behavior of the flies and noted that after 5-10 seconds, a preference could not be clearly determined. Flies would either remain in the T-maze entrance or would not have enough time to become adjusted to the light. Alternatively, after 30-60 seconds, the flies would often become inactive or stationary. Thus at the end of the 20 seconds, the distance travelled by *D. melanogaster* in centimeters from the centre of the T-maze was recorded. A total of 30 replicates were recorded for each of the wild type and *ort*$^{1}$ mutants. For each replicate, we rotated the T-maze to switch the red and blue films using a randomized coin flipping method. This was important to ensure that no external variables would affect our results.

For the purpose of our study, specimens that did not move from the centre of the T-maze were not used in the statistical analysis, and a t-test was performed only on specimens which moved towards blue side. This method was used so that distances traveled towards one side would not decrease or mask the effect of distances traveled towards the opposite side, as would happen if negative and positive values were used for distances traveled from the centre. In addition, a t-test was not calculated for specimens which moved towards the red side as the sample size for that population was too small (n=2 for wild type, and n=5 for mutants).

At the end of the experiment we recorded our data in tables, and calculated the mean, standard deviation, and 95% confidence intervals. Next, we applied the student's t-test to the data to determine if there was a statistical difference in the mean distance towards blue light between wild-type and the mutant flies. Despite the fact that our data were not normally distributed, we chose to use the t-test for the purposes of this course. The mean distances traveled

towards the blue side by the wild type and ort$^{1}$ mutants were plotted with 95% confidence intervals as depicted in Figure 3.

**Results**

The mean distances with 95% confidence intervals wild-type and *ort*$^{1}$ mutant *D. melanogaster* travelled towards blue were 4.2 ± 0.5 cm and 3.5 ± 0.7 cm respectively (Figure 3). The wild-type population had a sample size of 22, and the *ort*$^{1}$ mutants had a sample size of 18 after removal of specimens that did not move from the centre or went towards the red light. Wild type and *ort*$^{1}$ mutants were not significantly different in the distances they traveled towards the blue light ($p=0.11 > 0.05$). It was also found that a greater proportion of *D. melanogaster* moved towards the blue light (22 wild type, 18 *ort*$^{1}$ mutant) than towards the red light (n= 2 wild type, 5 mutant).

Throughout the experiment, we observed that-wild-type *D. melanogaster* expressed higher excitability and activity in regards to their movement in both the vial and the T-maze than *ort*$^{1}$ mutant *D. melanogaster*. Wild-type *Drosophila* appeared to move faster and flew more often inside the T-maze. In contrast, mutant *Drosophila* moved much more slowly, and used their legs rather than wings to move inside the T-maze.

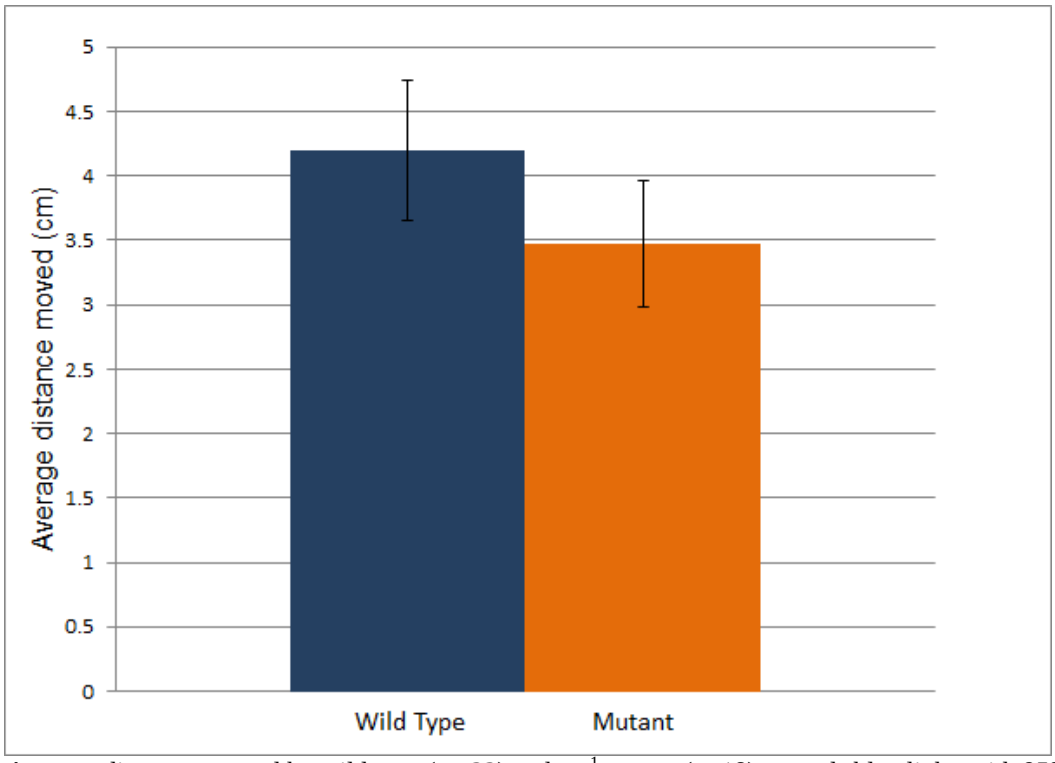

Figure 3. Average distances moved by wild type (n= 22) and *ort*[1] mutant (n=18) towards blue light, with 95% confidence intervals shown as vertical bars.

**Discussion**

From the results we found a general trend that more *D. melanogaster* moved towards the blue light than the red light. Of 30 wild-type specimens, 22 moved towards blue, 6 did not move from the centre, and only 2 moved towards red. Similarly, out of 30 mutant specimens, 18 traveled towards blue, 7 did not move, and only 5 traveled towards the red side. As *D. melanogaster* are color-blind for red light, both wild type and *ort*[1] mutants would not be able to perceive the side with only red light. Also, as Keene *et al.* (2011) found, adult *D. melanogaster* can perceive blue light and generally exhibit greater movement towards brightly lit environments as opposed to dim places, so this finding is consistent with what we had expected.

A t-test of the mean distances travelled by wild-type and *ort*[1] mutant *D. melanogaster* towards the blue light yielded a p-value of 0.11 (>critical value of 0.05). This demonstrates that

wild-type and mutant *D. melanogaster* were not statistically significantly different in the average distances they travelled towards the blue light. From this, we failed to reject our null hypothesis, and could not provide support for our alternate hypothesis. Contrary to what we thought in the introduction, wild-type and *ort*$^{1}$ mutant *D. melanogaster* did not differ significantly in the distances they traveled towards the blue light. This is surprising as the lack of outer R1-R6 photoreceptors in *ort*$^{1}$ mutants were expected to have some effect on their behaviour with regards to sensing light. It is possible that the lack of outer photoreceptors do not affect how bright colored lights appear. Recently, a study by Vinayak *et al.* (2013) found a positive correlation in *D. melanogaster* between light intensity and the length of time neurons linked to the visual system spend depolarized, so the number of photoreceptor cells may not be crucial in sensing light intensity so long as a sufficient number are functioning. However, it is important to note that this finding may be a result of our procedure not being thorough enough to detect a difference in the distances they traveled.

Our experiment started with 30 samples each for wild type and *ort*$^{1}$ mutants. However, because we had to omit the specimens that did not move from the centre of the maze, and the ones that moved towards red, the population size used for statistical analysis was reduced (see methods). As such, the final sample size was reduced to 22 for wild type, and 18 for mutants (as noted in the results). Thus, further experiments on this topic should be done to verify or disprove the results of our study. A replication of this experiment would greatly benefit from increasing the sample size, to ensure the results of a t-test are more accurate and reliable.

A further source of experimental error that could have contributed to uncertainty in our experiment may have occurred as a result of exposure to increasing temperatures and poor ventilation in the room where the experiment took place (as noted in the results). On the first day

of the experiment, over the course of three hours, the two 60 watt lamps we used to illuminate the colored films caused the room to heat up to temperatures higher than room temperature. Though the precise room temperature was not measured at the time, research has shown that increased temperatures can lead to decreased levels of motor activity and sprint speed in *D. melanogaster* due to a detrimental effect on enzyme function (Dillon *et al*. 2006, Dillon *et al.* 2009). Therefore this may have decreased the distances traveled by the flies towards the end of the experiment. We also noticed that the wild-type flies remained more active in their vials in comparison to the mutant flies and therefore may have been less affected by above optimal temperatures than mutant flies. On the second day of the experiment (during mutant trials), a fan was brought into the room, and the lamps were periodically turned off and the room was allowed to cool to minimize this error.

**Conclusion**

The results of this study demonstrated that both wild-type and *ort*$^{1}$ mutant *D. melanogaster* do not differ in the average distances they travel towards blue light. Thus, we failed to reject our null hypothesis, nor could we provide support for our alternate hypothesis. We interpreted this finding as meaning the *ort*$^{1}$ mutation does not affect *D. melanogaster's* perception of light intensity. However our experiment had several problems such as a small sample size, and an increasing room temperature which could have caused us to be unable to discern the difference in distances traveled by wild-type and o*rt*$^{1}$ mutant *D. melanogaster.*

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