Effects of light intensity on preferred environment of wild-type and *ort*^{1} mutant *Drosophila melanogaster*

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Abstract

Drosophila melanogaster have photoreceptors in their eyes that are responsible for sensing light. Their phototransduction system is similar to that of vertebrates, making them a useful model organism. Our experiment aims to determine the difference in light intensity preference between wild-type and *ort*^{1} mutant *D. melanogaster*. We used a T-tube and covered each arm with a different material, simulating different light intensity environments. We placed individual replicates in the T-tube and observed the time spent in each light intensity environment. Using the Kruskal-Wallis statistical test, we found that the wild-type *D. melanogaster* spent significantly more time in the lowest light intensity (38.0 ± 8.8 seconds) compared to bright light intensity (14.5 ± 7.6 seconds) and medium light intensity (7.5 ± 5.6 seconds), with a p value less than 0.0001. The *ort*^{1} mutant *D. melanogaster* did not spend a significantly different amount of time in any one light intensity environment, as the p value was 0.6737. Therefore, we support our hypothesis that light intensity has an effect on the time spent by wild type in areas of different light intensities while there is no effect on the time spent by *ort*^{1} mutants in areas of different light intensities. Our results suggest that *ort*^{1} mutants are unable to demonstrate a light intensity preference and that the *hclA* gene is necessary for detecting light and displaying a light preference in *D. melanogaster*.

Introduction

The eye of *Drosophila melanogaster,* commonly known as the fruit fly, serves many important functions relating to locomotion, behaviour, learning and most importantly the reception of visual sensory information (Von Lintig *et al*. 2001; Ofstad *et al*. 2011; Rieger *et al*. 2007). Photoreceptors are specialized neurons in the eye that are sensitive to light and initiate phototransduction, the process by which light is converted into electrical signals in the retina (UBC Biology 455 Course Notes, 2016, pers. comm.). There are many similarities in the phototransduction system of *D. melanogaster* and vertebrates, as such, they serve as a popular model system for electrophysiological, genetic and molecular biology studies about the mechanisms behind visual processing (Von Lintig *et al*. 2001). Understanding of the

phototransduction cascade in *D. melanogaster* can provide us with a better understanding of our own neural circuitry (Von Lintig *et al*. 2001).

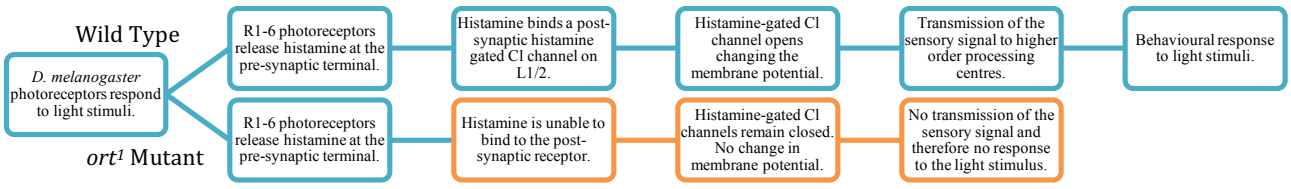

Figure 1: Model of the phototransduction response in *D. melanogaster.*

Electrophysiological experiments demonstrate that histamine-mediated synaptic transmission between a major class of photoreceptors, R1-6, and large laminar monopolar neurons, L1/2, is necessary in the *D. melanogaster* phototransduction cascade (Gengs *et al.* 2002; Pantazis *et al.* 2008; Rieger *et al.* 2007). The histamine receptors on L1/2 neurons are chloride channels (Gengs *et al.* 2002; Pantazis *et al.* 2008; Rieger *et al.* 2007). Subjects that lack the ability to produce histamine or have a mutation in the gene encoding the histamine-gated chloride channel demonstrate defective phototransduction abilities, as seen by a lack of current in large laminar monopolar neurons upon stimulation (Gengs *et al.* 2002; Pantazis *et al.* 2008; Rieger *et al.* 2007).

Iovchev *et al.* (2002) identified a 569 nucleotide deletion in the coding sequence of the *hclA* gene locus that produced a mutant phenotype known as ort^1. The *hclA* gene encodes a protein subunit of the L1/2 histamine-gated chloride channel (Gengs *et al*. 2002). The ort^1 mutants have defective synaptic transmission between photoreceptors R1-6 and L1/2, which impedes the transmission of sensory information to higher processing centres (Gengs *et al.* 2002; Pantazis *et al.* 2008; Rieger *et al.* 2007).

Studies investigating different photoreceptive mutants are needed to understand the roles of different genes and photoreceptive components of locomotive activity and behaviour at different light intensities (Rieger *et al.* 2007). The objective of our research was to gain insight into the light intensity preference of wild-type *D. melanogaster* and observe if the preference is maintained in ort^1 mutants. A measure of preference was established by recording time spent in areas of different light intensity. To realize our objectives, we worked with two sets of hypotheses:

H_{o1}: Light intensity has no effect on the time spent by wild-type *Drosophila melanogaster* in areas of different light intensities.

H_{a1}: Light intensity has an effect on the time spent by wild-type *Drosophila melanogaster* in areas of different light intensities.

H_{o2}: Light intensity has no effect on the time spent by ort^1 mutant *Drosophila melanogaster* in areas of different light intensities.

H_{a2}: Light intensity has an effect on the time spent by ort^1 mutant *Drosophila melanogaster* in areas of different light intensities.

We predicted that light intensity would have an effect on the time spent in areas of different light intensity by wild-type *D. melanogaster*. Further, we predicted that wild-type *D. melanogaster* would spend significantly more time in low light intensity environments, thereby implying a light intensity preference. The natural light intensity range of *D. melanogaster* is 0 to 100,000 Lux, but it has been shown that when given a choice of environments, *D. melanogaster* selected a low light intensity environment (5-10 Lux) for resting, grooming, feeding and locomotive activity (Gong *et al.* 2010; Rieger *et al.* 2007). In contrast, we predicted that light intensity would not have an effect on the time spent in areas of different light intensity by ort^1

mutants due to the nature of the mutation. As a result, we predicted that *ort*^{1} mutants would exhibit no light intensity preference and would spend equal amounts of time in all light intensities.

Methods

Our experiment occurred in two phases. In the first phase, we worked with wild-type *D. melanogaster* and in phase two we worked with *ort*^{1} *D. melanogaster*. The experimental set-up and procedure was the same in both phases.

We modified the arms of a glass T-tube such that each arm represented either a bright, medium or low light intensity environment. One arm of the T-tube was left unaltered and exposed to room light intensity, representing the bright environment. To create an environment with medium light intensity, we wrapped one arm of the T-tube in two layers of cheesecloth. We wrapped the remaining arm of the T-tube with aluminum foil to create a dark (low light intensity) environment. We secured the cheesecloth, aluminum foil and open ends of the T-tube with Parafilm (Figure 2).

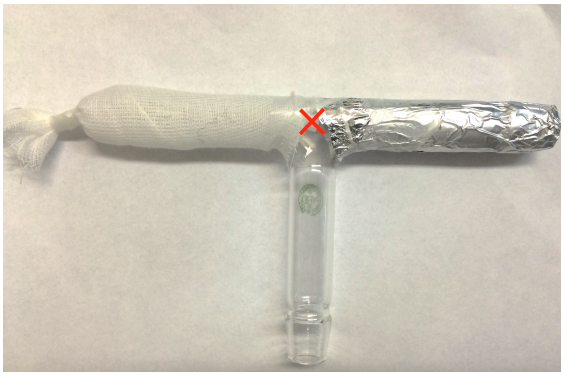

Figure 2: Experimental T-tube apparatus. The X represents the position of *D. melanogaster* at time=0.

To measure the light intensity in each area of the T-tube, we placed a light meter in open light, under two layers of cheesecloth, or under aluminum foil to mimic the T-tube environment. We made the assumption that the glass of the T-tube had a negligible effect on the light intensity inside the tube. We measured the light intensity of each area, as well as the ambient temperature

prior to every replicate. This allowed us to calculate the average light intensity of each environment over the course of the experiment. The mean light intensities for the low, medium, and bright environments were 3±0 Lux, 468±13 Lux, and 889±14 Lux, respectively. Room temperature was constant at 23℃.

We obtained four separate vials of *D. melanogaster* labelled 1, 2, 3, and 4, each containing approximately 20 adults. We anaesthetized the subjects in vial 1 by exposing them to CO_2 for 10 seconds. Following CO_2 exposure, we used forceps to quickly transfer 10 subjects, into individual test tubes for recovery. Recovery was defined as movements similar to those prior to anaesthesia. We gave subjects six minutes to recover from anaesthesia in the test tube. Subjects that did not recover were discarded.

After the six-minute recovery period, we transferred a single individual into the T-tube by inverting the recovery tube and connecting the open end to the open end of the bright arm of the T-tube, which was positioned vertically. Replicates were placed in the tubes individually to avoid any possible social interactions. We encouraged the replicate to move to position X in the T-tube, indicated on Figure 2, by gently tapping on the test-tube walls.

Once the replicate reached position X, we placed the T-tube in a horizontal position, started a one-minute timer and recorded the time spent by the replicate in each area of light intensity. We also made qualitative observations about the movement of the replicates. If the replicate did not move within 30 seconds of reaching position X, recorded values were not included in statistical analysis. If the replicate stayed at position X for less than 30 seconds and then moved, the time spent at position X was recorded as time spent in bright light intensity. After the one-minute observation period, we disposed of the replicate. We repeated this process for each replicate from vial 1. For each replicate, we rotated the orientation of the T-tube to

eliminate directional bias. We then repeated the entire process three times using subjects from the remaining vials.

We compiled the data for all replicates into Excel and graphed the data using the mean amount of time spent in dark, medium, and bright light intensities for wild type and mutants. In Excel, we calculated significant differences and 95% confidence intervals for each mean value to account for variation in our data. Next, we performed a Kruskal-Wallis statistical analysis test on the data using an online generator to obtain *p* values. This test was chosen as our data was non-parametric. We determined the significance of our results based on the calculated *p* value.

Results

Figure 3 shows the mean amount of time that wild-type and ort^1 mutant *D. melanogaster* spent in low (3±0 Lux), medium (468±13 Lux), and bright (889±14 Lux) light intensities. Wild-type *D. melanogaster* spent more time in the bright light intensity (14.5 ± 7.6 seconds) than in the medium light intensity (7.5 ± 5.6 seconds); however, they spent the most amount of time in the low light intensity (38.0 ± 8.8 seconds). The mean amount of time that wild-type *D. melanogaster* spent in different light intensities was significantly different as $p<0.0001$ which is less than 0.05.

Mutant *D. melanogaster* spent 17.6 ± 9.1 seconds in low, 22.17 ± 9.2 seconds in medium and 20.2 ± 8.9 seconds in bright light intensity environments. These results were not significantly different as our *p* value was 0.6737, which is greater than 0.05.

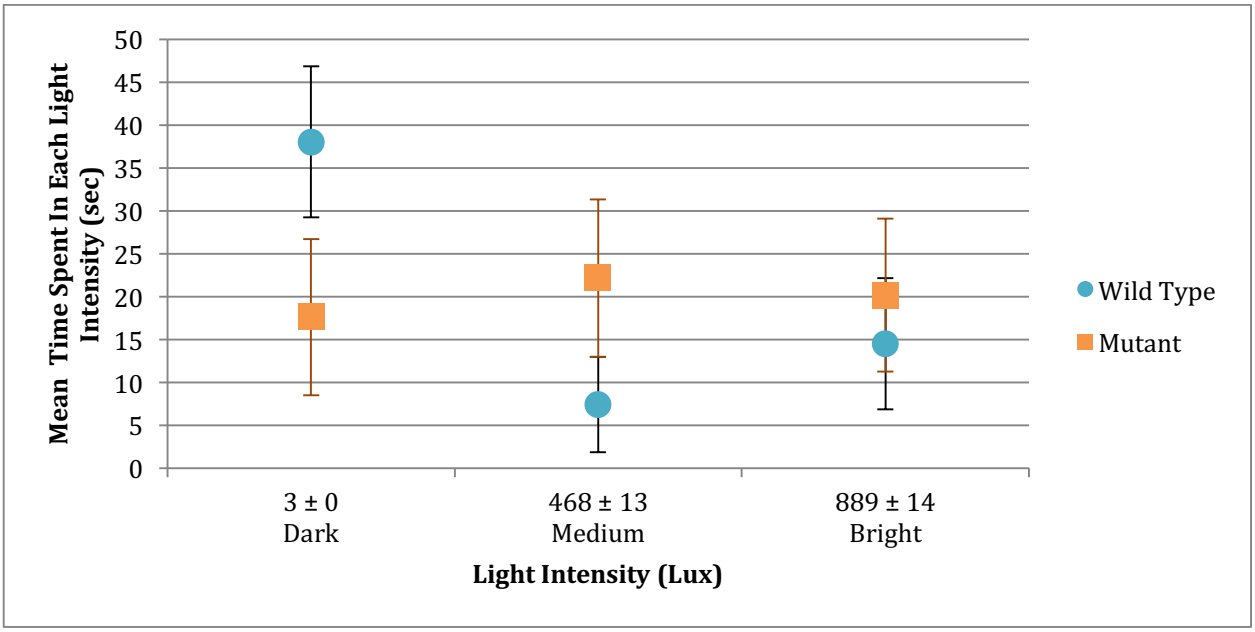

Figure 3: Mean amount of time *D. melanogaster* spent in varying light intensities. Wild type n= 33. *ort*^{1} mutant n= 35. The error bars represent 95% confidence intervals. There is significant difference in mean time spent in each light intensity environment in wild-type *D. melanogaster, p*<0.0001. There is no significant difference in mean time spent in each light intensity in mutant *D. melanogaster, p*=0.6737.

Discussion

Upon analysis of the time that wild-type *D. melanogaster* spent in each light intensity environment, the Kruskal-Wallis test gives a *p* value less than 0.0001, which is less than 0.05. We reject our first null hypothesis and provide support for our alternate hypothesis, as there is a significant difference in the time spent by wild-type *D. melanogaster* in different light intensity environments. This implies that wild-type *D. melanogaster* have a light intensity preference and provides support for our prediction that the time spent by wild-type *D. melanogaster* in different environments would be affected by light intensity. Conversely, upon analysis of the time spent by *ort*^{1} mutant *D. melanogaster*, the Kruskal-Wallis test gives a *p* value equal to 0.6737, which is greater than 0.05. We fail to reject our second null hypothesis as there is no significant difference in the time spent by *ort*^{1} mutant *D. melanogaster* in the different light intensity environments. This is consistent with our prediction that the time spent by the *ort*^{1} mutant would not vary among light intensities, and that *ort*^{1} mutants did not have a light intensity preference.

The *ort*^{1} mutation is a null mutation in the *hclA* gene; this mutation affects the ability of *D. melanogaster* to exhibit a light intensity preference (Pantazis *et al*. 2008; Gengs *et al*. 2002). The *hclA* gene encodes a protein subunit of a histamine-mediated chloride channel that is required for photoreceptor synaptic transmission, which allows *D. melanogaster* to detect different light intensities (Gengs *et al.* 2002). The *ort*^{1} mutation prevents *D. melanogaster* from making functional proteins required for photoreceptor synaptic transmission, thus, the channel can no longer interact with histamine (Iovchev *et al*. 2002; Pantazis *et al*. 2008; Gengs *et al*. 2002). As the *ort*^{1} mutants do not exhibit a light intensity preference, we suggest that the *hclA* gene is necessary for exhibiting light preference in *D. melanogaster.*

As described in our model (Figure 1), the wild-type *D. melanogaster* can detect different light intensities, thus they are able to move to their preferred light intensity. On the other hand, the *ort*^{1} mutants are unable to sense different light intensities and do not exhibit a preference. The *ort*^{1} mutants may have a preference, but perhaps they are unable to act on this preference because they cannot properly phototransduce (Gengs *et al*. 2002).

Our results with wild-type *D. melanogaster* are supported by Rieger *et al.* (2007) who found that wild-type *D. melanogaster* have a preference for light intensities around 5-10 Lux when resting, grooming, feeding and in locomotive activity. The ability to detect light and move to a preferred intensity may be beneficial to survival (Von Lintig *et al*. 2001; Ofstad *et al*. 2011). Therefore, if the *ort*^{1} mutant is unable to detect and react to varying light intensities, they may have complications with locomotion, behaviour, and learning but most importantly the reception of visual sensory information (Von Lintig *et al*. 2001; Ofstad *et al*. 2011; Rieger *et al*. 2007).

Precautions were taken in order to minimize any sources of error and variation in our experiment, despite our efforts they are still likely to be present. Research shows that female *D.*

melanogaster may have a prolonged recovery time from CO_2 anaesthesia (Iovchev *et al.* 2002). This source of biological variation may serve as a source of error in our study as we used both male and female *D. melanogaster*.

Another possible source of error is the administration of CO_2. Given the experimental setup, the pressure at which gas was administered could not be precisely controlled, therefore, the concentration of CO_2 in each vial may have been slightly different. Increasing the exposure time or the concentration of CO_2 can result in a prolonged recovery time from anaesthesia (Iovchev *et al.* 2002; Nilson *et al.* 2006). While we were unable to control the concentration, we attempted to minimize this source of error by administering CO_2 for the same amount of time per vial. Additionally, we used four vials so that each replicate was only anaesthetized once.

Hong *et al*. (2006) report that temperature can have an effect on the recovery time from CO_2 anaesthesia in both wild-type and *ort*^{1} mutant *D. melanogaster*, however, the effects are more pronounced in the mutant strain. High temperatures result in a slower recovery time for the *ort*^{1} mutant and would therefore have influenced their ability to move in the T-tube apparatus (Hong *et al.* 2006). To minimize this error, we ensured a constant room temperature throughout the experiment.

Conclusion

Wild-type *D. melanogaster* spent significantly more time in low light intensity, thus we reject our first null hypothesis and provide support for the alternate hypothesis. The *ort*^{1} mutant *D. melanogaster* did not spend a significant amount of time in any light intensity, thus we fail to reject our second null hypothesis. Our results support our predictions and suggest that wild-type *D. melanogaster* prefer darker environments and *ort*^{1} mutants do not exhibit a light intensity preference.

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