Wild-type and *ort*^{1} mutant *Drosophila melanogaster* spend a varying amount of time in different light intensities

Sharon Bassan, B. Iselle Flores Ruiz, Komal Virk, Ying Shi Yuan

Abstract

Drosophila melanogaster have photoreceptors in their eyes that allow them to respond to different light intensities. The *ort*^{1} mutants have these photoreceptors, but are missing the R1-R6 rhabdomeres in them. This mutation disrupts the communication between the organisms' lamina cells and photoreceptors. The focus of our experiment was to compare the proportion of time spent in different light intensities in the wild-type and *ort*^{1} mutant *D. melanogaster*. We tested two light intensity treatments in the arms of a T-maze: dim light intensity ($\leq$ 0.05 lux) and high light intensity (> 0.05 lux). We then recorded the time that 40 wild type and 40 *ort*^{1} mutants spent at each light intensity over 30 seconds. Our hypothesis was that light intensity will affect the time spent under varying light conditions in the wild type and *ort*^{1} mutants of *Drosophila melanogaster*. We found that 73% of wild-type flies spent more than 15 seconds in the dim light location, while only 5% of *ort*^{1} mutants preferred staying in the dim light arm of the T-maze. We used Fisher's exact test to analyse our data, and found the difference in choice between the wild type and *ort*^{1} mutants to be statistically significant (p = 0.0001). The lack of R1-R6 rhabdomeres affects the *ort*^{1} mutants' ability to detect dim light and relay the information to the brain and this may cause the flies to ignore low light regions.

Introduction

Drosophila organisms have apposition eyes that contain eight different photoreceptors per optical unit (Borst 2009). Photoreceptors R7 and R8 are located in the middle and are surrounded by photoreceptors R1-6 (Borst 2009). The R1-6 photoreceptors are responsible for detecting movements and low light intensities, whereas R7 and R8 regulate positive phototaxis (movement towards light) (Gong 2012). Each photoreceptor cell has an organelle, known as a rhabdomere. Rhabdomeres contain rhodopsin, a photopigment that senses light (Gong 2012; Borst 2009). R1-6 cells are connected to the lamina (Figure 1), which filters signals to other neurons (Borst 2009). Histamine is the neurotransmitter responsible for connecting R1-6 and the

lamina neurons via axon terminal communication inside the lamina (Figure 1) (Borst 2009; Fischbach and Dittrich 1989).

Light passes through the photoreceptors creating an image from one specific point of view (Fischbach and Dittrich 1989). Subsequently, the signal passes through different neurons beginning from the lamina to the medulla to the lobula and finally to the lobula plate (Figure 1) (Fischbach and Dittrich 1989). Lastly, columnar neurons connect the eye to the central brain (Fischbach and Dittrich 1989).

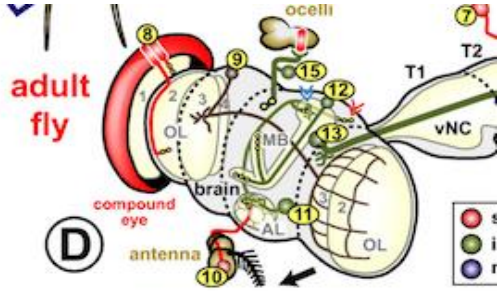

Figure 1. Image by Sanchez-Soriano *et al.* (2007). Adult *Drosophila*'s eyes are divided into four nervous areas: the lamina (number 1), medulla (number 2), lobula (number 3) and lobula plate (number 4) (Fischbach and Dittrich 1989; Sanchez-Soriano *et al.* 2007).

Investigators studying *Drosophila* vision commonly mutate different genes to understand how they are linked to specific visual processing tasks (Borst 2009). One of them is an *ort*^{1} mutant (Iovchev *et al*. 2002; Oh *et al*. 2013). *D. melanogaster* flies with this mutation lack R1-R6 rhabdomeres in the photoreceptors (Harris *et al.* 1976; O'Tousa *et al.* 1989). This mutation results in communication problems between the lamina cells and the photoreceptors (O'Tousa *et al.* 1989).

D. melanogaster is a popular model organism because their genetic information, intercellular communication and behaviour are similar to that of humans (Wixon and O'Kane 2000). Thus, they allow us to study these characteristics in order to better comprehend those of humans (Wixon and O'Kane 2000).

Although scientists have studied *D. melanogaster* extensively to learn more about their visual anatomy and physiology, and their resemblance to humans, they have done little research on their behaviour towards visual stimuli (Borst 2009). Our research objective was to provide more insight regarding the difference between light intensity preference in wild-type and ort^1 mutant flies. In addition, we wanted to further understand how the ort^1 mutation might interfere with *Drosophila*'s ability to sense light. Our null hypothesis was that light intensity will not have an effect on the amount of time spent in the different T-maze arms by the wild type and the ort^1 mutant of *Drosophila melanogaster*. Our alternative hypothesis was that light intensity will have an effect on the amount of time spent in the different T-maze arms by the wild type and the ort^1 mutant of *Drosophila melanogaster*.

D. melanogaster is naturally subjected to light intensities ranging from 0 to 100,000 lux (Rieger *et al.* 2007). It is known that *Drosophila* spp. have different light preferences depending on their life stage (Rieger *et al.* 2007). For example, larvae experience negative phototaxis, meaning that they move away from light while adult flies experience positive phototaxis (Gong 2012). However, studies such as Rieger *et al.* (2007) have found that wild-type, adult *D. melanogaster* prefer low light intensities of about 5 to 10 lux for resting. Hence, *Drosophila* prefer dim light areas for leisure activities (Rieger *et al.* 2007). As previously discussed, ort^1 mutants lack the gene responsible for having efficient connections between the photoreceptors in charge of detecting dim light (Gong 2012). For this reason, we predicted that wild-type *Drosophila* would spend more time under low light conditions than the amount of time they spend under high light conditions. Conversely, due to the mutation in ort^1 flies, we predicted that the mutants would spend the majority of time under high light conditions due to their inability to detect low light (Gong 2012).

Materials and Methods

Before beginning our experiment, we brought 90 wild-type *Drosophila* and 90 *ort*^{1} mutant *Drosophila* into room light intensity for 60 minutes to acclimatize them. We used a T-maze apparatus that consisted of three arms to create three distinct light intensity environments (Figure 2A). The first arm of the apparatus was wrapped in aluminium foil and received an average light intensity of 0.002±0.00 lux. The second arm was exposed to room light intensity of an average of 506±38.31 lux, and the third arm was exposed to a high light intensity of an average of 7408±163.99 lux by shining the light from a 60-Watt light bulb on the arm (Figure 2B). The variance of the light intensities was calculated based on the average light intensity of each arm. This light bulb was positioned 10 cm above the apparatus. We closed each arm of the apparatus with a cotton ball to prevent the flies from leaving. We used a piece of cardboard measuring 15 cm by 15 cm to block the dim light intensity arm and room light intensity arm from being exposed to the light from the 60-Watt light bulb. Then, we marked the place on the apparatus where each of the light intensity regions began. The centre region of the apparatus, which was not assigned a light intensity value, is where the flies were placed to start the experiment. Each of the four experimenters set up the T-maze apparatus horizontally to avoid any gravity bias.

We then rolled a die to randomly select the orientation of the T-maze for each replicate. To bring a fly into the apparatus for each trial, we placed one end of a straw in a vial filled with wild-type flies and the other end of the straw was brought to the centre region of the apparatus through the room light intensity arm. We waited for the fly to travel through the straw to the centre of the apparatus. When the fly was at the centre of the apparatus, the room light intensity arm was closed with a cotton ball, the 60-Watt light bulb was turned on and the timer was started

for 30 seconds. We chose the set time to be 30 seconds based on the approximate experimental length of previous studies such as Iovchev *et al*. (2002) and Yamaguchi *et al.* (2010). We measured the amount of time the fly spent in each arm and recorded these values. The criterion for data collection was that the fly must move to at least one of the arms. Therefore, if a fly did not move to one of the arms, that replicate was discarded. We observed and recorded the order in which the fly visited the arms. After 30 seconds, we turned the 60-Watt light bulb off between replicates to prevent a temperature change of the high light intensity arm and discarded the used replicate into a separate flask. These steps were repeated for 39 additional wild-type and 40 *ort*^{1} mutant replicates.

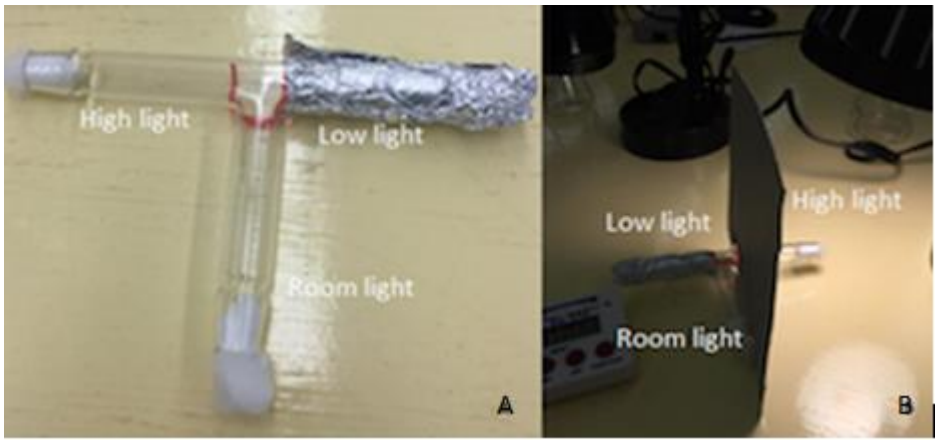

Figure 2. A. T-maze apparatus used to create three light intensity environments. **B.** Experimental setup showing the arms of the T-maze exposed to different light intensities. We placed a 60-Watt light bulb above one arm of the apparatus to create the high light intensity region. A piece of cardboard was included in the setup to prevent light from the light bulb from shining on the low light and room light regions of the apparatus.

For our statistical analysis we used Fisher's exact test. In our analysis, we compared the time spent in the dark location ($\leq$ 0.5 lux) to the time spent in the light locations (> 0.5 lux). We combined the data of the time spent in room light intensity and high light intensity because very few flies spent time in the room light intensity.

Results

Qualitative observations show that wild-type *D. melanogaster* were more active than the *ort*^{1} mutants. The wild-type *D. melanogaster* showed a great deal of movement both in the vials and the T-maze by flying and climbing up the walls. During the experiment, they readily selected a light intensity. In addition to this, they showed signs of being alert and explored their surroundings. However, the *ort*^{1} mutants were not very active in the vials, staying at the bottom. When placed in the T-maze, the *ort*^{1} mutants that selected a light intensity would often go to the ceiling of the T-maze arm and remain there.

To determine whether the wild type and *ort*^{1} mutants preferred dark locations compared to light locations, we calculated the number of replicates that spent 15 seconds or more in the dark location. As seen in Figure 3, the wild type spent a larger proportion of time in the dark location (n=40, mean=18.7, SD= 11.7) than the *ort*^{1} mutants (n=40, mean=1.63, SD= 6.64). The *p*-value calculated from Fisher's exact test, p = 0.0001, is less than the critical value (p = 0.05). Of the 40 wild-type *D. melanogaster* used in the data analysis, 29 replicates spent 15 seconds or more in dark conditions (73%), while only 2 out of 40 *ort*^{1} replicates spent more than 15 seconds in dark conditions (5%). Most of the *ort*^{1} mutants stayed out of the dark location, with the exception of two replicates, contributing to the large error bars in Figure 3. The wildtype seem to have preferred the dark location over the light location, as there were 11 replicates that did not spend more than 15 seconds in the dark location (27%).

There were 5 wild type and 22 *ort*^{1} mutants that did not make a selection. These flies did not leave the centre region of the apparatus. We did not include these replicates when analysing our data because they did not meet the criteria of moving from the centre region and spending time in any of the three light-intensity regions.

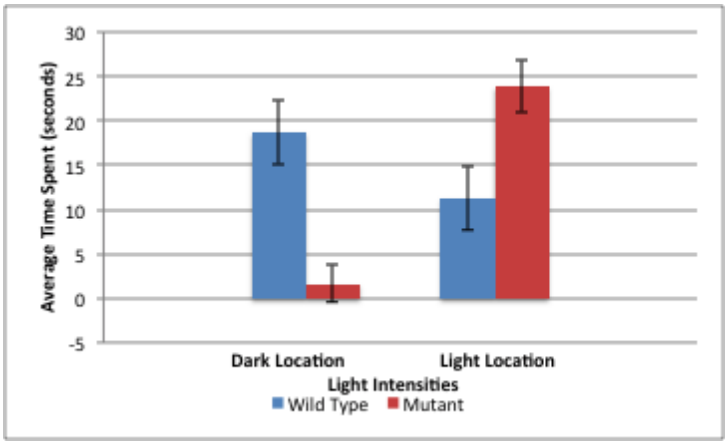

Figure 3. The frequency of wild-type and *ort*^{1} mutant *D. melanogaster* which preferred various light intensities; the dark (≤ 0.5 lux) and light locations (> 0.5 lux). The vertical error bars represent 95% confidence intervals, n=40 for wild type and n=40 for *ort*^{1} mutants, *p*-value = 0.0001 calculated from Fisher's exact test.

The 95% confidence intervals for wild type were found to be 18.7±3.62 seconds for the dark location and 11.3±3.62 seconds for the light location. For the *ort*^{1} mutants, the 95% confidence intervals calculated were 1.63±2.06 seconds for the dark location and 23.9±9.81 seconds for the light location. The 95% confidence intervals for each of the wild-type samples were the same, whereas they were different for the *ort*^{1} mutant.

Discussion

According to the results of the Fisher's exact test, we reject the null and provide support for the alternative hypothesis because the *p*-value of 0.0001 was less than 0.05. This indicates that the wild-type and mutant *D. melanogaster* were significantly different in the proportion of time that they spent in the dark region.

Our results agree with our prediction that wild-type *Drosophila* would spend more time under dark light conditions than under light conditions (Figure 3). Rieger *et al*. (2007) found wild-type *Drosophila* prefer to remain in dark areas when engaged in resting activities. Since our experiment only involved placing one *Drosophila* at a time into a T-maze, with light being the only stimulus, our results agree with those of Rieger *et al.* (2007). As aforementioned, wild-type

Drosophila have functional R1-6 photoreceptors that can differentiate among light intensities (Gong 2012). Thus, their optical units correctly send histamine neurotransmitters to the lamina cells, cascading the optical signal until it reaches the brain (Fischbach and Dittrich 1989).

As stated in our observations, wild-type *Drosophila* were active both in the vials and inside the T-maze. Sclichting *et al.* (2014) and Bachleitner *et al*. (2007) mention that wild-type flies show greater activity levels under higher light intensities. The explanation for this is that wild-type *Drosophila*'s' activity levels are lower under low light intensities because they cannot see as well as under higher light intensities (Bachleitner *et al*. 2007).

In comparison, rhodopsin-lacking mutant *Drosophila* are less active under the same conditions (Sclichting *et al.* 2014). They concluded that the discrepancy in activity levels is due to photoreceptor mutations, perhaps in the lack of rhodopsin in these cells. Flies lacking photopigments in the photoreceptors R1-6 cannot sense different light intensities (Sclichting *et al.* 2014). Thus, their activity levels are lower than the wild-type flies that are able to sense these light differences.

From the trend we see in Figure 3, the mutant *Drosophila* spent a larger proportion of time in the light location, just as we had predicted. Since the R1- R6 photoreceptors have been known to be responsible for detecting dim light (Gong 2012), any problem in the communication between R1-R6 photoreceptors and the lamina cells in mutants may result in the inability to sense the low light intensity. As a result, unlike the wild-type, which tend to move to dark light conditions (Rieger *et al.* 2007), the mutant flies did not have an intention of moving to the dark light location, they ignored the low light arm in the T-maze. Moreover, the results of Gao *et al.* (2008) suggest that ort^{1} mutant flies need to be under higher light intensity to have a phototaxic

response towards light compared to wild-type flies. This may indicate that mutant flies have an intention to move to a location with higher light intensity.

One source of uncertainty in our experiment was the potential temperature rise in the high light intensity arm due to the irradiance from the 60-Watt light bulb. However, we tried to minimize this uncertainty by turning off the light bulb in between each replicate. Although we did not measure the temperature throughout our experiment, we did not observe a trend regarding the flies avoiding the high light intensity arm as the experiment progressed. Thus, it is likely that the increase in temperature was not significant enough to affect the movement of the flies to the high light intensity region.

Conclusion

Our project focused on comparing the light intensity preference between wild-type and ort^1 mutant *Drosophila melanogaster*. Upon statistical analysis of the data collected using Fisher's exact test, our results indicated that the difference in time spent in the dark location between the wild-type and ort^1 mutant of *D. melanogaster* was statistically significant because the *p*-value of 0.0001 was lower than 0.05. Further research looking at the time spent in different light intensities by other mutant strains of *D. melanogaster* could be conducted to better understand the effects of light intensity on behaviour in *Drosophila.*

Acknowledgements

We would like to thank our instructor, Carol Pollock, for guiding us through our project and providing us with ample assistance to conduct our research. We would also like to acknowledge our lab technician, Mindy Chow in her role in helping us learn lab techniques and ensuring our experiment was conducted in an effective scientific manner. In addition, we would

like to thank both Carol and Mindy for their suggestions about how to conduct our statistical analysis. Our Teaching Assistant, Nicole Gladish had a large role in the design and implementation of our project. We would like to thank her for her advice in helping us refine our experiment and providing us with support throughout the course. Furthermore, we would like to acknowledge the work of our anonymous peer reviewers for their valuable input and suggestions. Lastly, we would like to thank the University of British Columbia for providing us with the opportunity and resources to conduct biological research.

Literature Cited

Bachleitner, W., Kempinger, L., Wulbeck, C., Rieger, D., and Helfrich-Forster, C. 2007. Moonlight shifts the endogenous clock of *Drosophila melanogaster* [online]. PNAS, **104**(9): 3538-3543.

Borst, A. 2009. *Drosophila*'s view on insect vision [online]. Current Biology, **19(**1): R36-R47.

Fischbach, K. F., and Dittrich, A. P. M. 1989. The optic lobe of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structure. Cell and Tissue Research, **258**(3): 441-475.

Gao, S., Takemura, S., Ting C. Y., Huang, S., Lu Z., Luan, H., Rister, J., Thum, A. S., Yang, M., Hong S. T., Wang J. W., Odenwald, W. F., White B. H., Meinertzhagen, I. A., and Lee, C. H. 2008. The Neural Substrate of Spectral Preference in *Drosophila* [online]. Neuron, **60**(2): 328-342.

Gong, Z. F. 2012. Innate preference in *Drosophila melanogaster* [online]. Sci China Life Sci, **55**: 8–14.

Harris W. A., Stark W. S., and Walker J. A. 1976. Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. J. Physiol. **256**: 415–439.

Iovchev, M., Kodrov, P., Wolstenholme, A. J., Pak, W. L., and Semenov, E.P. 2002. Altered drug resistance and recovery from paralysis in *Drosophila melanogaster* with a deficient histamine-gated chloride channel. Journal of Neurogenetics, **16**(4): 249-261.

O'Tousa, J. E., Leonard, D. S., and Pak, W.L. 1989. Morphological defects in oraJK84 photoreceptors caused by mutation in R1-6 opsin gene of *Drosophila* [online]. Journal of Neurogenetics, **6**(1): 41-52.

Oh, Y., Jang, D., Sonn, J. Y., and Choe, J. 2013. Histamine-HisCl1 receptor axis regulates wake-promoting signals in *Drosophila melanogaster* [online]. PLoS ONE, **8**(7): e68269.

Rieger, D., Fraunholz, C., Popp, J., Bichler, D., Dittmann, R., and Helfrich-Forster, H. 2007. The Fruit Fly *Drosophila melanogaster* Favors Dim Light and Times Its Activity Peaks to Early Dawn and Late Dusk [online]. Journal of Biological Rhythms, **22**(5): 387-399.

Sclichting, M., Grebler, R., Peschel, N., Yoshii, T., and Helfrich-Forster, C. 2014. Moonlight Detection by *Drosophila*'s Endogenous Clock Depends on Multiple Photopigments in the Compound Eyes [online]. Journal of Biological Rhythms, **29**(2): 75-86.

Yamaguchi, S., Desplan, C., and Heisenberg, M. 2010. Contribution of photoreceptor subtypes to spectral wavelength preference in *Drosophila* [online]. Proceedings of the National Academy of Sciences of the United States of America, **107**(12): 5634-5639.

Wixon, J., and O'Kane, C. 2000. Featured organism: *Drosophila melanogaster.* Yeast, **17**(2): 146-153.