The effect of different light colours on the phototaxis response of wild-type and *ort*^{1} mutant *Drosophila melanogaster*

Julia Johnson, Ori Nevares, Manmeet Sandhu, Rikul Thapar

Abstract

Drosophila melanogaster is a small organism commonly known as the fruit fly. Little research has been carried out on the effect of *ort*^{1} mutations, which are known to cause defects in synaptic transmission of the R7 and R8 photoreceptors in *D. melanogaster*. These photoreceptors are normally sensitive to blue and green light, and therefore *ort*^{1} mutants have impaired vision for these colours of light. The objective of this study was to observe the effect of the *ort*^{1} mutation and light colour on the phototaxis response in mutant and wild-type Oregon-R strains of *D. melanogaster*. This was tested by administering each *D. melanogaster* into a T-shaped test tube apparatus, which had a coloured filter at the top and a clear area at the bottom. The time each replicate spent in the top or bottom of the apparatus was recorded. Mutant and wild-type were tested in blue, green, and white (control) light treatments. Although it was found that the wild-type *D. melanogaster* spent more time in the coloured area as compared to *ort*^{1} mutants, the results were not statistically significant ($p>0.05$). However, the proportion of time spent in each colour was significantly different between each colour treatment ($p<0.05$).

Introduction

Iovchev *et al.* (2002) identified that a deletion mutation in *hclA* gene that produced a phenotype known as *ort*^{1} with defects in synaptic transmission between photoreceptors R7 and R8 (Gengs *et al*. 2002). The *hclA* gene codes for a histamine-gated chloride-channel subunit, which plays a major role in controlling the phototaxis response and maintaining vision in *D. melanogaster* (Gengs *et al.* 2002). It has been observed that a decrease in histamine concentration alters the phototaxis response amongst *D. melanogaster*, which confirms that histamine is a major neurotransmitter in the visual system of *D. melanogaster* (Iovchev *et al.* 2002). When wild-type flies are stimulated (e.g., by tapping the culture vial), they are observed to instantly fly upward toward a light source, which constitutes a positive phototaxis and negative geotaxis response (Melzig *et al.* 1996). However, because the *ort*^{1} mutation reduces the release of the important neurotransmitter histamine, it is predicted that there will be a reduced phototaxis response among the mutants as compared to wild type. Many of the previous studies

that have involved *D. melanogaster ort*^{1} mutants have examined the effects of light intensity as measured by distance travelled, but there are a limited number that have focused on phototaxis. Thus, this study observes the phototaxis response of wild-type and *ort*^{1} mutant *D. melanogaster* in different colour wavelengths and provides an opportunity to increase our understanding of how wild-type and mutant *D. melanogaster* respond to light stimuli and apply this knowledge to how they behave in their natural environments.

Initially, we tested if light colour has an effect on the phototaxis response of *D. melanogaster*. H_{01}: The colour of the light filter has no effect on the proportion of time *D. melanogaster* spend in each section of the test tube apparatus; while the alternative, H_{A1}: Light filter colour has an effect on the proportion of time *D. melanogaster* spend in each section of the test tube apparatus. Next, we predicted that the mutation caused by a deletion in the *hclA* gene for the *ort*^{1} mutant strain should limit the number of flies that entered regions of the test tube that were covered with green and blue filters. Thus, H_{02}: The presence of the mutation, *ort*^{1}, has no effect on the proportion of time *D. melanogaster* spend in each section of the test tube apparatus; while H_{A2} : The presence of the mutation, *ort*^{1}, has an effect on the proportion of time *D. melanogaster* spend in each section of the test tube apparatus. Also, we determined whether there was an interaction between the two variables, *ort*^{1} mutation and light filter colour. Thus, H_{03}: There is no interaction between filter colour and the presence of the *ort*^{1} mutation in their effect on the proportion of time *D. melanogaster* spend in each section of the test tube apparatus. H_{A3}: There is an interaction between filter colour and the presence of the *ort*^{1} mutation in their effect on the proportion of time *D. melanogaster* spend in each section of the apparatus.

Methods

We obtained approximately 50 mutant and 50 wild-type flies to carry out the experiment. First, we anesthetized the flies with CO_2 gas in their containers and transferred them into separate test tubes using forceps and paint brushes. We allowed the flies about five minutes to recover from the gas. Organisms that did not show signs of proper recovery were discarded. Proper recovery was defined as walking and flying around when the organism's test tubes were tapped, similar to their behaviour prior to being anesthetized.

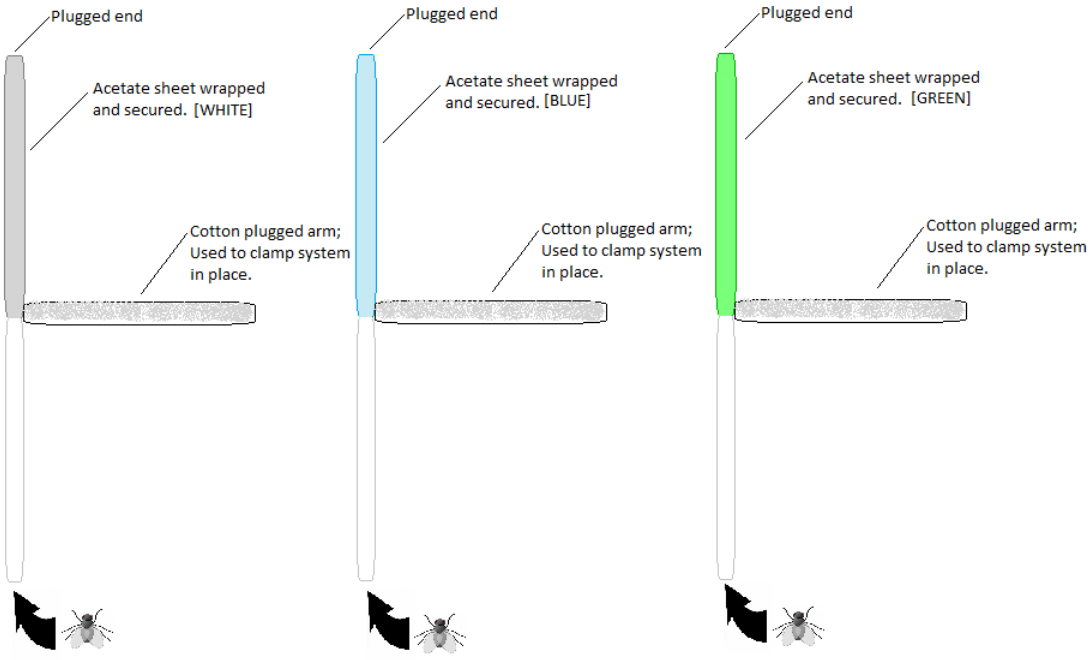

Figure 1. Diagram of apparatus used with the coloured area on the top and clear area on the bottom. Light intensity was maintained at 150 lux for the upper portions of the apparatus.

We used a T-shaped glass test tube as our experimental apparatus, as seen in Figure 1. We blocked off the horizontal arm of the test tube with a cotton ball so that the organism could not enter it. The top arm was wrapped with a colour filter. The length of each arm of the tube was 60 mm. We made the blue and green filters from plastic acetate sheets cut to size whereas cheese cloth was used to make the white (control) filter. Cheese cloth was used to reduce the light intensity and ensured it was approximately the same between the different colour filters.

We used a light meter to measure the light intensity prior to placing the flies in the apparatus. The light intensities were measured as: 151 lux for the blue filter, 164 lux for the green filter, and 175 lux for the white filter. We attached the apparatus to a clamp stand in the vertical position to ensure the flies would enter due to their natural tendency to fly upwards. The fly was then released out of its test tube and allowed to enter the apparatus. The fly was allotted a time of 30 seconds inside the apparatus, which we found in previous literature as an appropriate time for the flies to adjust and respond to the colour differences (Ali *et al.* 2011). We recorded the amount of time each replicate spent in each section of the test tube. After each replicate was concluded, the organism was discarded. No organisms were reused. A total of 92 individual flies were used throughout the procedure, as 8 out of the 100 flies did not recover properly. We analyzed the results for statistical significance using a two-way analysis of variance (ANOVA) test.

Results

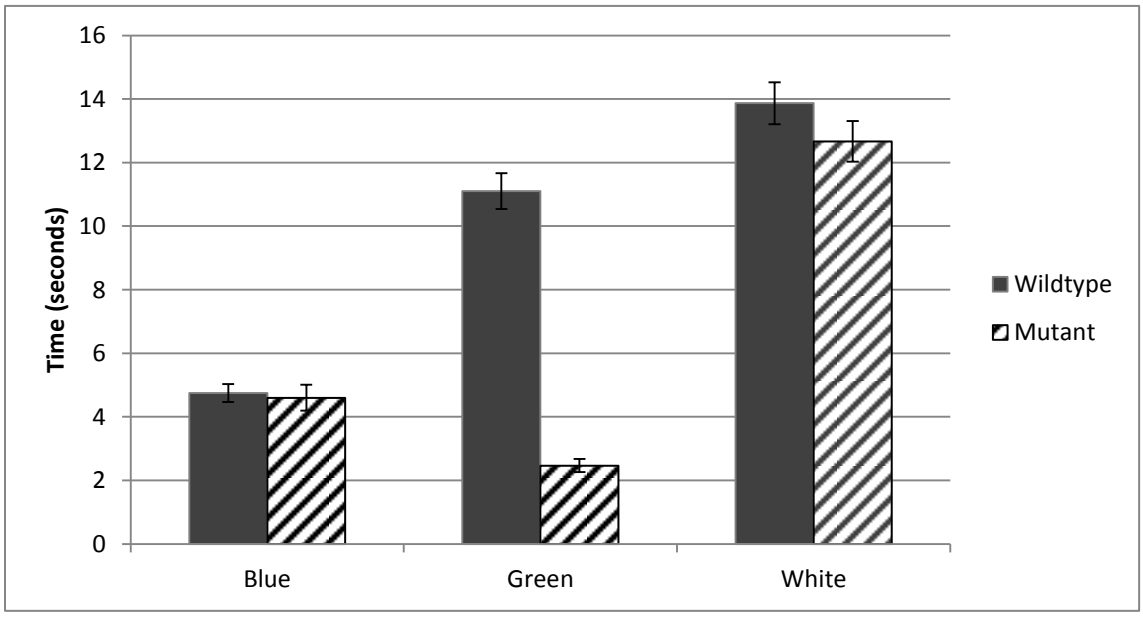

Figure 2. Mean time spent by *Drosophila melanogaster* in the coloured section of the apparatus. Data is separated by colour and strain type. 95% Confidence intervals are calculated using the standard error of the mean. n=20, p=4.3117x10^{-4} between treatments.

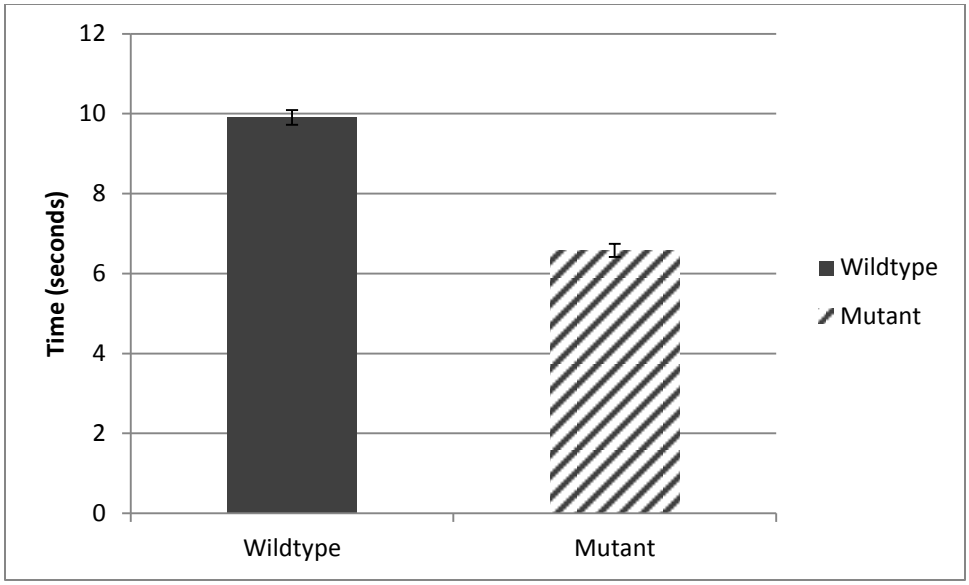

Figure 3. Mean time spent by *Drosophila melanogaster* in the coloured section of the apparatus separated by organism type. 95% confidence intervals are calculated using standard error of the mean. n=20, p=6.6277x10$^{-2.}$

Based on the results of the two-way ANOVA test, we obtained a p-value of 6.628x10^{-2} for the effect of organism type (mutant or wild-type). The p-value for the effect of the different filter colours was 4.310x10^{-4} and the p-value for the interaction between these two variables was 1.140x10^{-1}. As shown in Figure 2, overall both wild-type and mutant *D. melanogaster* spent the greatest proportion of time, about 13 seconds, in the white filter. Wild-type *D. melanogaster* spent more time in the green filter than the blue filter, approximately 11 seconds, whereas *ort*^{1} *D. melanogaster* spent more time in the blue filter than the green filter, about 4 seconds. Figure 3 shows overall, wild-type *D. melanogaster* spent more time than mutants in the coloured sections of the apparatus. Error bars were calculated using standard error of the mean.

Discussion

Based on the results from the two-way ANOVA test, we rejected our null hypothesis that the colour of the filter has no effect on the proportion of time spent by *D. melanogaster* in the T-tube apparatus because our calculated p-value supports 95% confidence (p < than the critical

value of 0.05). Thus, we were able to provide support for the alternative hypothesis, H_{A1}. However, we were unable to reject H_{02}: the presence of the mutation, *ort*^{1}, has no effect on the proportion of time *D. melanogaster* spend in each section of the test tube apparatus, since the *p*-value was found to be greater than a critical value of 0.05, and therefore we failed to provide support for H_{A2}. We also failed to reject H_{03}: there is no interaction between filter colour and the presence of the *ort*^{1} mutation in their effect on the proportion of time the flies spend in each section of the apparatus. We therefore failed to support H_{A3}, because the calculated *p*-value was greater than 0.05, and there was substantial overlap between the 95% confidence intervals of the mean time spent by mutant and wild-type flies in the coloured section of the apparatus, as seen in Figure 2. The results provide some support for our prediction, that *D. melanogaster* with the *ort*^{1} mutation should have a reduced phototaxis response in blue light and green light. This is because mutant flies were observed to spend less time in the green-coloured section of the apparatus than wild type, but were not consistent with our prediction under blue light, where the mutant flies spent about the same proportion of time as the wild type, as seen in Figure 2.

As mentioned earlier, the R7 and R8 photoreceptors present in the retina of *D. melanogaster* are sensitive to wavelengths of UV light (100 nm - 400 nm) and blue (450 nm - 495 nm) or green (495 nm - 570 nm) light, respectively (Morante and Desplan 2008). There are also two histamine gated chloride channels, known as ort, which are responsible for relaying the sensory input from the photoreceptors to higher order neurons for further processing (Gao *et al.* 2008). Therefore, mutations in the gene *hclA*, which codes for the histamine gated channels, have been previously noted to cause a decrease in the phototaxis response of *D. melanogaster* to UV and green light because the sensory signals from the photoreceptors cannot be processed (Gao *et al.* 2008). This can be a possible explanation for why the *ort*^{1} mutant flies spent a significantly smaller proportion of time in the green-coloured section of the apparatus than the wild type

throughout the course of our experiment. The results we obtained hold some similarities with those found by other researchers who also studied the *ort*^{1} mutation. For example, Gao *et al.* (2008) found that *ort*^{1} mutant *D. melanogaster* flies exhibited a phototaxis response to green light which was two orders of magnitude weaker than the response of wild type flies. Gao *et al.* (2008) also propose that in many insects, it is common to find a preference for specific wavelengths of light over others. Often, green light is viewed as indicating the presence of available food, such as in water fleas. This may also explain why *D. melanogaster* has a high attraction to green light (Storz and Paul 1998). Figure 2 shows that we obtained similar results, as wild-type flies significantly spent more time in the green light compared to *ort*^{1} mutant flies. It is unclear, however, based on these results that this is due to a preference for green light over blue light, or a discrepancy that may be caused by sources of error within the experiment. One source of error that may have impacted the proportion of time recorded is variation in the activity level of the flies. The first set of anesthetized flies were transferred into an empty test tube using forceps, which may have caused more anatomical damage to their wings or legs compared to the second set of flies who were pulled out using a paint brush. Although we proceeded to only use flies that showed adequate signs of recovery, such as walking or flying, some anatomical damage may still have occurred which we could not see. The flies with greater physical damage may have been less active than the flies which did not sustain any injuries. Qualitative observations recorded during the experiment revealed that some flies were quicker to walk into the apparatus than others who circled near the base of the apparatus. Therefore, it may be that a larger proportion of the less active replicates were used with wild type for blue light than green light, impacting the results. In addition, the test tube needed to be inverted or tapped in an attempt to get the fly at the bottom in preparation to transfer it to the apparatus. At times, the forceful tapping may have caused shock to the flies and thus increased the time they took to begin

walking upwards in the test tube, which may contribute to a greater time spent in the bottom (clear) arm of the apparatus.

The findings of several experiments with *D. melanogaster* that lacked functional R1-6 photoreceptor cells bring up the importance of the influence of light intensity on the phototaxis response (Washington 2010). The *Rh-1* mutants were found to be more strongly attracted to blue light than green light, in the majority of light intensities used during data collection. Although we tried to minimize the impact of light intensity by measuring it and ensuring it was approximately similar for all of the colour filters, prior to starting our experiment, it may have still fluctuated during the course of the experiment. As we stood in front of the apparatus to carry out the protocol, we may have lowered the light intensity of the filter by shading it. This could have been avoided by measuring the light intensity after each use of the apparatus, and confirming it was similar to its starting value. R1-6 photoreceptors, also present in the retina of *D. melanogaster*, are important for colour vision in *D. melanogaster*. Therefore, the results obtained by Washington (2010) also hold some similarity to the ones we obtained, as the mutants we used also seem to display a preference for blue light over green light, which can be seen in Figure 2.

Other sources of uncertainty may also have impacted the results we obtained. One is the age of the *D. melanogaster* flies, which was not known to us. Older flies may have a different preference for light colour than flies at larval stages. Also, although we saw a trend in which wild-type *D. melanogaster* spent, overall, more time in the coloured section of the apparatus, the *p*-value we obtained for the different light colour treatments failed to give us statistically significant results. However, our *p*-value, 6.628×10^{-2} was not very far from the critical value of 0.05. We may have obtained statistically significant results if we had used a larger number of

replicates and hence a larger sample size. Finally, using a larger sample size would have further minimized the role of sex interactions, as mentioned earlier.

Conclusion

Light colour was found to have an effect on the phototaxis response of wild-type and *ort*^{1} mutant *D. melanogaster*, as measured by the proportion of time spent in the coloured versus clear sections of the experimental apparatus. As predicted wild-type flies spent more time in green light than blue, while the opposite was true for the mutants. This trait may be motivated by an instinctual need to find food. We failed, however, to find a significant effect for organism type (mutant or wild-type) on the phototaxis response. We also failed to support our prediction that the presence of the *ort*^{1} mutation would cause a decrease in the phototaxis response of *D. melanogaster* in blue light and green light. In addition, we did not find a significant interaction between light colour and organism type in influencing the phototaxis response of *D. melanogaster*.

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