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Melanin in the Suppression of Blue/UV Light Induced Retinal Degeneration in *Drosophila melanogaster*

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ABSTRACT

The leading cause of visual impairment is age-related macular degeneration (AMD), an irreversible eye disorder that causes permanent blindness. AMD is projected to rise from 196 million to 288 million cases worldwide by 2040. AMD can be caused by high-energy light exposure, which leads to oxidative damage that deforms pigment cells protecting the retina. Like humans, *Drosophila melanogaster* contain varying amounts of retinal pigment. The purpose and novelty of this research is to ascertain the efficacy of various pigments against blue and UV light, and the relative severity of each light on the retina. Flies were exposed to blue and UV light to induce retinal degeneration, which was quantitatively measured through the FLEYE software by analyzing the irregularity of eye units. Results suggest that more severe retinal degeneration is caused by blue light, followed by UV, then white light across white eyes, red eyes, and sepia eyes (significant p-values of 0.00005, 0.00090, and 0.00014, respectively), and that white eyes undergo the most degeneration when exposed to blue or UV light, followed by red eyes, then sepia eyes (significant p-values of 0.04710 and 0.04765, respectively). However, pigment did not make a significant difference for flies under white light (p-value of 0.06420). Future work could investigate genes or antioxidant supplementation as potential treatments for AMD. This research provides insight into the prominence of high-energy light in inducing retinal degeneration, and the potential for retinal melanin in preventing it, improving the currently limited understanding of AMD.

Introduction

Significance of Age-Related Macular Degeneration (AMD)

Human eyesight is progressively degrading, with the leading cause of visual impairment attributed to age-related macular degeneration (AMD). This irreversible eye disorder leads to permanent blindness in advanced stages and can increase mortality risk by 40%. Since the exact pathogenesis of AMD is still somewhat unclear, it is not completely understood why the eye disorder is associated with a higher risk of mortality. Oxidative stress could spread to other neurons, potentially causing neurodegenerative diseases like Alzheimer's which decrease life expectancy. Additionally, visual impairment from the late stages of AMD could lead to psychological issues and functional difficulties that increase the chances of accidental injuries, consequently raising mortality (Zhu et. al, 2018).

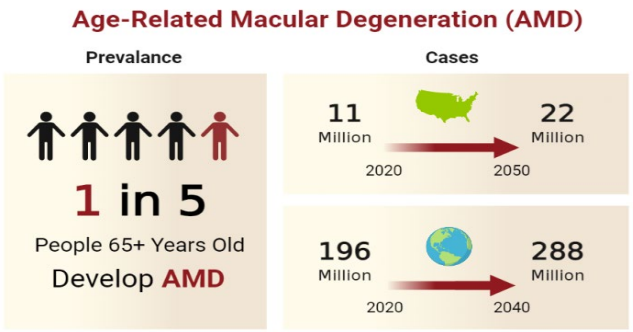


Figure 1. Current Prevalence and Future Projections of Age-Related Macular Degeneration.

Pathogenesis of Age-Related Macular Degeneration (AMD)

AMD causes the deterioration of the macula, which is a thin part of the retina located in the back of the eye, that accounts for both central vision and color vision. The retina contains several integral cells and structures that are essential for vision, but are negatively impacted during the progression of AMD. For example, photo-receptor cells, which are responsible for detecting light, are maintained by the overlying layer of retinal pigment epithelium cells (RPE). RPE cells produce and store melanin, the natural pigment that helps protect eyes from harmful light by absorbing it. As depicted in Figure 2, RPE cells undergo deformation during AMD, which not only reduces the amount of melanin produced to protect the eye, but also causes damage to the neighboring photoreceptor cells, contributing to vision loss (Hadziahmetovic & Malek, 2021).

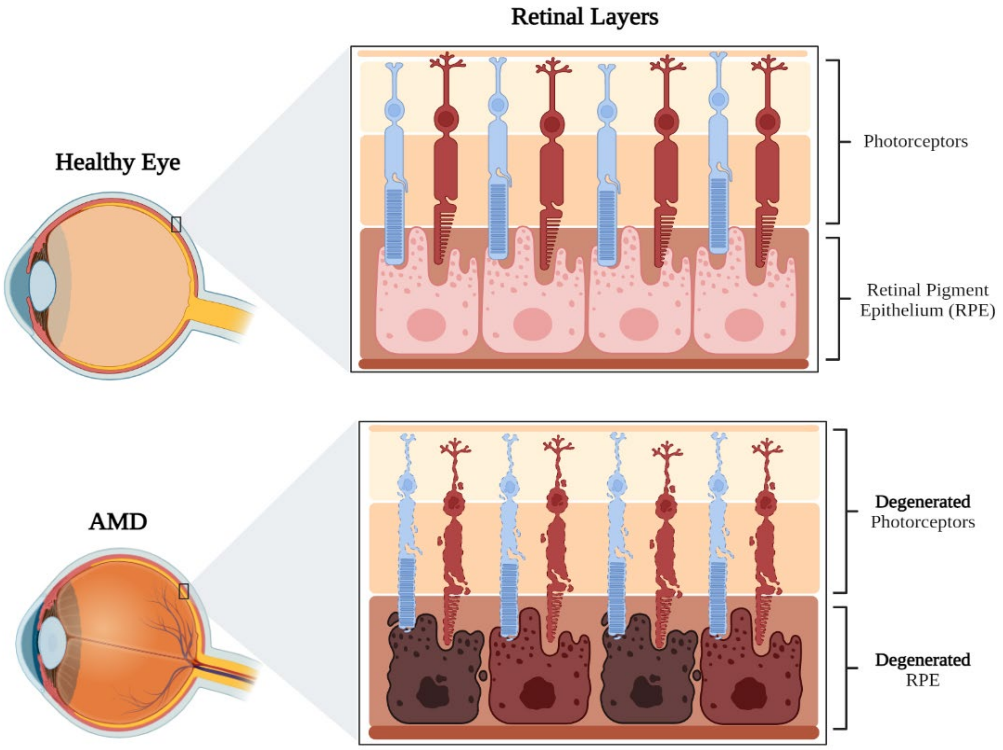


Figure 2. Comparison of Retinal Layers in a Healthy Eye versus in an Eye with AMD. The healthy eye has normal photoreceptors and normal retinal pigment epithelium cells. The eye with AMD has degenerated photoreceptors and degenerated retinal pigment epithelium cells.

Research revealed that oxidative stress is directly linked to AMD. Oxidative stress is a biochemical process in which reactive oxygen species (ROS) and antioxidants are imbalanced. ROS are very reactive since they contain an unpaired electron, so antioxidants can neutralize them by donating an electron without becoming unstable themselves. One way oxidative stress is caused is through prolonged light exposure. Light of shorter wavelengths and higher energy—such as ultraviolet (UV) and blue light—causes considerable oxidative damage to the eye. The sun is a primary source of blue and UV light, and the blue light emitted from electronic screens can also contribute to oxidative stress over time, especially in the eye since the retina is vulnerable to oxidative stress. ROS results as a byproduct when oxygen is metabolized in the mitochondria, and the retina—which is where the macula, photoreceptors, and RPE cells are located—is susceptible to oxygen metabolism, because it consumes high levels of oxygen. Additionally, the retina is almost constantly exposed to both sunlight and artificial light, so external sources of ROS (blue and UV light) cause cell death after being absorbed by RPE. The risk of eye issues like AMD is increased when RPE absorb high-energy light, because one of the main factors of the eye disorder is the deformity and/or degeneration of the RPE cells that produce and store melanin (Figure 2). This pigment decreases as people age, so the macula and photoreceptors become more vulnerable to damage. In addition, with age comes a decrease in antioxidants that neutralize ROS and protect the eye from oxidative stress (Saccá et al., 2018).

A specific type of oxidative stress present in the eye is lipid peroxidation, in which membrane lipids undergo oxidative damage. Lipids are organic molecules that do not dissolve in water, and lipid peroxides (peroxides are compounds containing oxygen) negatively alter the structure and function of membranes. In the retina, RPE cells act as a membrane to photoreceptors, so RPE deformation compromises the regular activity

of photoreceptors. Once lipid peroxidation is initiated, it spreads rapidly in a chain of self-propagation, making it very difficult to stop the progression of AMD. The main pathway necessary for light-induced lipid peroxidation to occur is an intact phototransduction cascade. During phototransduction, the conversion of light changes the electrical potential across a cell membrane, such as when blue light converts rhodopsin—a sensory protein in photoreceptors—into its active form. Phototransduction opens ion channels, causing the influx of Ca^{2+} into photoreceptors that directly increases oxidative stress in the retina. Therefore, blue light causes lipid peroxidation and leads to retinal degeneration that is difficult to halt (Chen et. al, 2017).

Drosophila Melanogaster as a Model Organism

My research aims to further study blue and UV light induced retinal degeneration using an *in vivo* model. *Drosophila* is an ideal model organism, because like humans, flies contain retinal pigment which determines eye color and the amount of protection from light-induced oxidative stress (Tomlinson, 2012). Furthermore, flies' relatively short life-spans allow eye disorders like AMD to develop faster, especially through light exposure (Zhu et al., 2017).

Previous research on *Drosophila* visual systems has revealed much about their compound eyes. As Figure 3 depicts, the *Drosophila* retina consists of single eye units called ommatidia, and each contains eight photoreceptors, special cells that detect light ranging from UV to green. Specific types of photoreceptors contain a light-sensitive pigment known as rhodopsin, which partially identifies with human rhodopsin found in melanocyte cells. Similar to RPE cells, melanocyte cells produce and store melanin. Mutations in rhodopsin can cause retinal degeneration in both humans and flies, and the mosaic structure of photoreceptors with rhodopsin in fly retinas is very similar to that in human retinas, which suggests that certain aspects of *Drosophila* vision, including color vision, is analogous to human vision (Zhu, 2013).

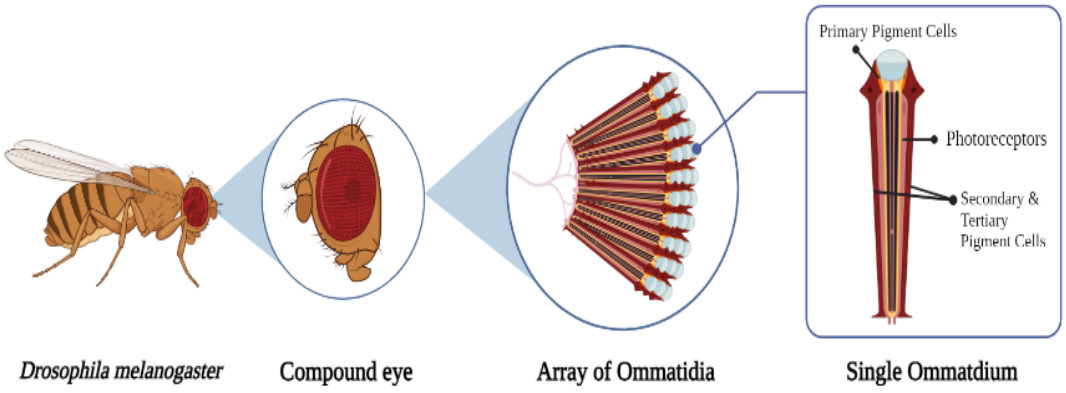


Figure 3. The Structure of *Drosophila* Compound Eye and Ommatidia. The compound eye is made up of units called ommatidia, organized in a hexagonal array. Each individual ommatidium contains photoreceptors, as well as primary, secondary, and tertiary pigment cells.

To reiterate, prolonged blue and UV light exposure causes oxidative damage in the eye, especially in the more vulnerable retina; oxidative damage leads to the deformation of RPE cells and the neighboring photoreceptors, ultimately contributing to the progression of AMD, which is why retinal pigment could be a prominent way to protect against eye disorders by absorbing high-energy light (Hadziahmetovic & Malek, 2021; Saccá et al., 2018). In both humans and *Drosophila*, the pigment type and amount of pigment determines eye color. For instance, red-eyed flies have the pteridine pigment and sepia-eyed flies have the ommochrome pigment, while white-eyed flies lack both. Darker shades correspond to more pigment, so the *Drosophila* eyes with

least to most melanin are white, red, and sepia, which is analogous to how human eyes that are light blue have less melanin than dark brown eyes (Grant et. al., 2016).

Purpose, Novelty, Variables, and Hypothesis

The purpose of my research is to study the effect of retinal melanin in suppressing blue and UV light induced retinal degeneration in *Drosophila melanogaster* models with white eyes, red eyes, and sepia eyes, as the relative efficacy of all three eye colors in preventing degeneration is untested. Specifically, each distinct pigment type (pteridine and ommochrome) has a different pathway to produce its corresponding color (Grant et. al., 2016), and the way the different pathways impact the level of light absorption and the degree of retinal degeneration is currently untested. Furthermore, while there has been some research on the general effect of UV light on the eyes, most studies relevant to oxidative stress and retinal degeneration in Drosophila eyes focused only on blue light, which is why I plan to use both blue and UV light to provide a more realistic representation of the types of light human eyes are exposed to on a daily basis.

The two independent variables are the eye color and light type. I hypothesize that the flies with the most melanin (sepia-eyed) will undergo the least retinal degeneration, and those with the least melanin (white-eyed) will undergo the most, since retinal pigment is expected to absorb high energy light and prevent oxidative stress. Regarding the different light types, I hypothesize that the blue light will have a more severe effect than the UV, as blue light is known to penetrate the eye further, and the retina is located at the back of the eye (Zhao et. al., 2018).

The dependent variable of my research is the retinal degeneration in *Drosophila* eyes after exposure to blue and UV light. Retinal degeneration can be measured using the FLEYE software, an automated image analysis system that uses pictures of the external eye surface to quantitatively analyze the degree of degeneration. Since a degenerated retina causes the morphology of the eye to change, the software can detect bright spots that result in the photograph when the ommatidia reflect light. Therefore, the FLEYE method can accurately determine retinal degeneration levels using non-invasive methods, and is a freely available online software (Diez-Hermano et. al., 2015).

Methods

Materials

All fly stocks were obtained from Bloomington *Drosophila* Stock Center. The three fly models were Canton S. (red-eyed), se^{1} (sepia-eyed), and w^{1188} (white-eyed), with the stock numbers, #27215, #1668, and #3605, respectively.

Unless otherwise stated, all equipment and supplies necessary for fly maintenance and disposal, fly food preparation, CO_2 sorting, eye imaging, etc., were obtained from the science labs at the Academy of Science (AOS).

The blue light, UV light, and white light LED lamps were purchased from Amazon.com, with the following ASIN numbers, respectively: #B08B8NNS43, #B08QDF35S1, and #B08PJQG3B6. The Canon Leica Light Microscope with attached camera was used for eye imaging. The softwares used in this research were FLEYE (available from doi:10.1186/s13024-015-0005-z), Bio-Formats Importer (https://www.openmicroscopy.org/bio-formats/downloads/), and the Leica Application Suite X (https://www.leica-microsystems.com/products/microscope-software).

Culturing and Maintaining Flies

Flies were housed in plastic vials with foam flugs, and it was ensured that vials were not overpopulated. The temperature was maintained at room temperature (about 22°C), so they had approximately a two-week life cycle. When expanding stocks, flies were transferred to new vials by the tapping method every 4 days, and when they were not being used for experimentation, vials were tapped every 3 weeks. In order to dispose of flies, vials of flies were frozen at -20°C for at least 1 hour.

Fly Food Preparation

Using an electronic balance, scoopula, and weigh boats, the following masses of the dry ingredients were measured: 6.75 grams yeast, 3.90 grams soy flour, 28.50 grams yellow cornmeal, and 2.25 grams agar. A graduated cylinder was used to measure the masses of the wet ingredients: 30 mL light corn syrup and 390 mL distilled water. The wet and dry ingredients were combined in a 500mL beaker and thoroughly mixed together with a glass stirring rod. The beaker was put in the microwave for 30 second increments until the mixture started to boil; the glass stirring rod was used to stir the mixture in between boils to prevent an overflow. A thermometer was used to ensure the food cooled between 60°C and 70°C, before adding 1.88 mL of propionic acid (a mold inhibitor). Finally, the fly food was poured into several vials, which were then covered with a cheesecloth to prevent flies contaminating the food while it cooled to room temperature. Vials were regularly monitored to ensure that the food was not moldy or contaminated, and if necessary, the vials were appropriately disposed of (see Section 2.2).

CO_2 Sorting

Flies were sorted based on gender through CO_2 sorting. First, flies were anesthetized by inserting the nozzle of a CO_2 gun into the vial and exposing the flies to a gentle air flow of CO_2 gas. Once the flies were anesthetized, they were placed on a CO_2 pad under a light microscope. The flies were separated into two piles based on gender, using a sorting feather. Once sorting was completed, the flies were placed back into the vial, and the equipment was cleaned with ethanol wipes to prevent contamination during future use.

Light Exposure

Vials of *Drosophila* were placed inside Tupperware boxes; there was one box per light group (blue, UV, white light). One LED lamp was attached to each cardboard box, and each Tupperware box was placed in the cardboard box with the appropriate light. The boxes were covered with a dark cloth to prevent unwanted light from escaping or entering.

Experimental groups (flies exposed to blue or UV light) were first exposed to 8 hours of white light, followed by 4 hours of blue or UV light exposure, and 12 hours of darkness. Control groups were exposed to 12 hours of white light followed by 12 hours of darkness. Temperature was held constant at room temperature (about 22°C). Light exposure was continued from the larval stage to when adults emerged (approximately two weeks).

Imaging the Eye Surface

After the two-week period of light exposure, the eyes of newly-eclosed adult flies (around 1-day old) were imaged. First the Leica Application Suite X (LAS X) was downloaded on a computer. Then, the newly-eclosed

flies were anesthetized with CO_2 and sorted (see Section 2.4). Only the eyes of female flies were imaged for this research. Male flies were appropriately disposed of (see Section 2.2).

The eyes of the immobilized female flies were set parallel to the objective of the microscope, and illuminated with the microscope lights (light settings were held constant for all trials and groups). A computer was connected to the microscope camera with a USB cable, and the LAS X software was opened so that the flies under the microscope could be viewed live on the computer. Images of at least 10 eyes were taken for each group, with brightness set at 80% and a white balance performed on each image.

Image Processing & Statistical Analyses

First, the FLEYE macros, ImageJ plugin, and Bio-Formats Importer were downloaded (see *Materials* section).

The FLEYE region of interest (ROI) macro was run first, and the ImageJ "polygon" tool was used to select a region of interest on each image. The ROIs were saved on the ROI manager on ImageJ.

After selecting ROIs, several parameters were optimized on ImageJ: the pixel length was 1.85 micrometers, x-displacement for the filter was 5 pixels, the y-displacement for the filter was 0 pixels, the noise tolerance for the Find Maxima plugin was 5 pixels, and the grid cell width was 20 pixels. With these calibrated parameters, the Find Maxima plugin on ImageJ was executed to locate ommatidia within the ROI on each image. The selected maxima were filtered and converted into a black and white image with a subtracted background, and a squared grid overlay was applied to the image. The "line selector" tool on ImageJ was used to measure the distance between neighboring maxima. The distances were added as items on the ROI manager, and the ImageJ software generated measurements including the standard deviation of the lengths between neighboring maxima.

The standard deviation data from ImageJ was entered on Google Sheets, and an index of irregularity of the maxima distribution was calculated using a variable specified in the article that developed the FLEYE software: the logarithm of the variance of the mean nearest neighbor distances (of maxima).

Kruskal-Wallis statistical analyses were performed on the data to determine if there was a significant difference in levels of retinal degeneration between the flies with the same pigment under different light schedules, and the flies with different pigments under the same light schedules. The p-values that the tests yielded were used to determine significance of the differences in irregularity indices.

Safety Precautions

Closed-toed shoes were worn when dealing with glassware or chemicals. Lab coats, lab goggles, and gloves were worn when dealing with chemicals such as propionic acid, which can cause serious irritation if it gets in contact with the eyes or skin. When handling hot substances, hot hands or beaker tongs were used and goggles were worn to protect the skin and eyes. Exposure to blue and UV light was minimized by keeping the lamps in a closed setup (cardboard boxes). The flies were completely anesthetized before sorting or imaging their eyes to prevent escape or contamination.

Methodology Summary

Figure 4 below illustrates the three predominant methodologies in this research.

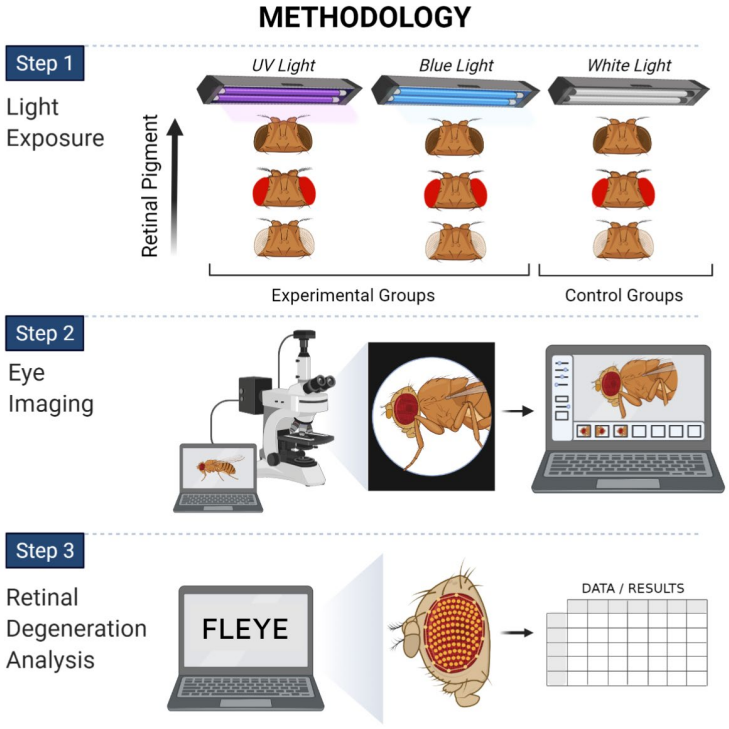


Figure 4. Summary of Methodology. Step 1 is to expose fly larvae to light until adults emerge. Step 2 is to image the eyes under a microscope. Step 3 is to process the images using FLEYE and analyze levels of retinal degeneration.

Results

Standard Deviations and Irregularity Indices

Table 1. Standard Deviations of the Distances Between Maxima per Trial for Each Group

	Blue Light Group			UV Light Group			White Light Group (Control)		
TRIAL	**White**	**Red**	**Sepia**	**White**	**Red**	**Sepia**	**White**	**Red**	**Sepia**
1	7.1687	4.362	2.57000	2.1921	5.3082	2.2620	1.3690	1.3273	1.2356
2	3.1543	2.4597	3.7455	3.2723	2.0927	3.2004	1.6059	0.8671	1.2143
3	2.4107	2.0642	2.3419	5.8801	5.7032	3.5596	1.4789	0.8502	0.4786
4	4.2636	1.5302	3.3021	2.4709	2.0086	2.4434	0.6535	1.2811	0.5297
5	5.9827	2.4707	3.2045	1.7798	1.5173	1.4549	1.2744	1.7312	0.6488
6	7.4922	5.9257	3.9711	3.5162	3.8855	1.3430	0.7645	1.7124	0.7159
7	1.9973	3.5133	2.1595	5.4802	2.0110	2.9433	1.1383	0.7838	0.6664

8	4.9688	3.1430	1.6340	3.5586	1.1441	1.7192	1.6343	0.5239	0.4489
9	4.5224	1.4992	3.2414	4.9949	3.0072	2.2830	1.1143	0.9224	1.4560
10	6.1192	4.3178	2.2509	6.2561	0.8409	0.6113	0.8717	0.7950	0.8148

Table 2. Irregularity Index[1] per Trial for Each Group

	Blue Light Group			**UV Light Group**			**White Light Group (Control)**		
TRIAL	**White**	**Red**	**Sepia**	**White**	**Red**	**Sepia**	**White**	**Red**	**Sepia**
1	1.7108	1.2790	0.8198	0.6817	1.4498	0.7088	0.2728	0.2459	0.1838
2	0.9978	0.7817	1.1470	1.0297	0.6414	1.0104	0.4114	-0.1238	0.1687
3	0.7642	0.6295	0.7391	1.5387	1.5122	1.1028	0.3398	-0.1409	-0.6401
4	1.2595	0.3694	1.0375	0.7857	0.6057	0.7759	-0.3695	0.2151	-0.5519
5	1.5538	0.7856	1.0115	0.5007	0.3621	0.3256	0.2106	0.4767	-0.3758
6	1.7492	1.5454	1.1978	1.0921	1.1788	0.2561	-0.2332	0.4672	-0.2903
7	0.6008	1.0914	0.6687	1.4775	0.6068	0.9376	0.1125	-0.2115	-0.3525
8	1.3925	0.9946	0.4265	1.1026	0.1169	0.4706	0.4266	-0.5615	-0.6957
9	1.3107	0.3517	1.0214	1.3971	0.9563	0.7170	0.0940	-0.0701	0.3263
10	1.5733	1.2705	0.7047	1.5926	-0.1505	-0.4275	-0.1191	-0.1993	-0.1779
Means	1.2913	0.9099	0.8774	1.1198	0.7280	0.5877	0.1145	0.0097	-0.2405

[1]Irregularity indices correspond to the level of retinal degeneration; a greater index represents more AMD, while a smaller index represents less AMD.

Sample Calculations

Sample Calculation #1: Irregularity Index

This calculation is for the irregularity index of Control S (Sepia-eyed under white light) Trial 1 (see Table 2 for the standard deviation and Table 3 for the calculated irregularity index).

$$irregularity\ index\ =\ log(\sigma^2)$$
$$irregularity\ index\ =\ log(1.2356^2)\ \approx\ 0.1838$$

Sample Calculation #3: Mean Irregularity Index

This calculation is for the mean index of irregularity of the Control S trials (see Table 3).

$$\frac{0.1838 + 0.1687 - 0.6401 - 0.5519 - 0.3758 - 0.2903 - 0.3758 - 0.2903 - 0.3525 - 0.6957 + 0.3263 - 0.1779}{10} \approx -0.2405$$

Kruskal-Wallis Statistical Test p-Values

Table 3. p-Values[1]: Comparing All 3 Pigments under the Same Light Schedule

p-Value	Groups
0.06420	White Light Schedule
0.04765	UV Light Schedule
0.04710	Blue Light Schedule

[1]p-values in bold are considered significant for being <0.5

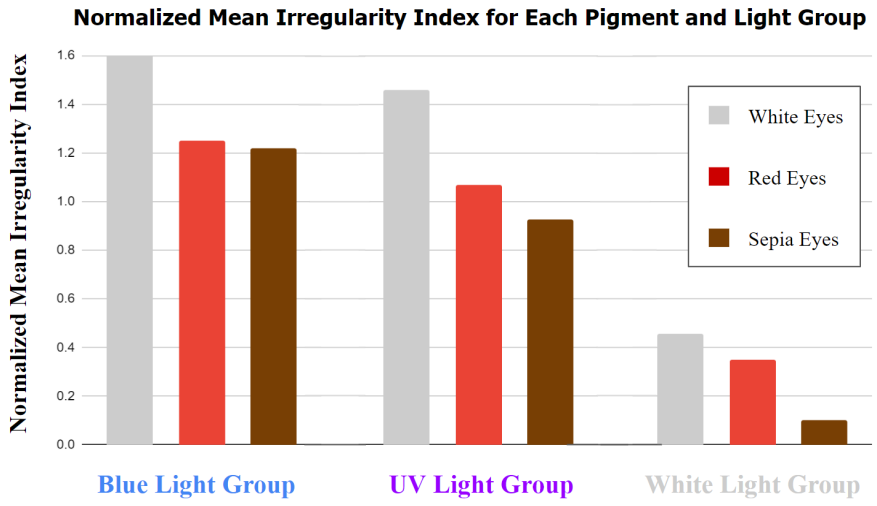
Table 4. p-Values[1]: Comparing All 3 Light Types for the Same Pigment

p-Value	Groups
0.00014	Sepia-Eyed Flies
0.00090	Red-Eyed Flies
0.00005	White-Eyed Flies

[1]p-values in bold are considered significant for being <0.5

Graph of Normalized Irregularity Indices

Graph 1. Normalized Mean Irregularity Index for Each Pigment and Light Group



Graph 1 depicts the normalized mean irregularity indices (shown on the y-axis) across all the groups. The white, red, and sepia bars represent the white-eyed flies, red-eyed flies, and sepia-eyed flies respectively, for each of the three light schedules (shown on the x-axis).

Review of Hypotheses, Results, and p-Values

It was hypothesized (see Section 1.4) that the flies with the most melanin (sepia-eyed) will undergo the least retinal degeneration, and the flies with the least melanin (white-eyed) will undergo the most degeneration. It was also hypothesized that blue light will have a more severe effect than the UV light in inducing retinal degeneration.

Graph 1 depicts the normalized mean irregularity indices, illustrating that the blue light group had the highest mean indices for each pigment, followed by UV and white light respectively. Additionally, the white-eyed flies had the highest mean indices, followed by red and sepia-eyed respectively, within each light type. Both of these trends are consistent with the hypotheses. The p-values from Kruskal Wallis tests (Table 4 and Table 5) indicate the statistical significance of the differences between irregularity indices, and primarily support the hypotheses.

For example, the p-values in Table 4 support the hypothesis that more pigment leads to less degeneration induced by *high-energy light*. There were statistically significant p-values for the comparison of irregularity indices for the different pigments under blue light (p-value 0.04710), as well as for the different pigments under UV light (p-value 0.04765). However, pigment did not make a significant difference when flies were exposed to white light, as the p-value of 0.06420 was above 0.05, and therefore not considered significant. These results suggest that the amount of melanin in the eyes does make a notable difference in the amount of degeneration *Drosophila* eyes undergo when they are exposed to blue or UV light, as the flies with the most pigment (sepia-eyed) underwent the least degeneration while the flies with the least pigment (white-eyed), underwent the most degeneration. Yet, even though the graph and the mean irregularity indices display the same trend regarding pigment for the flies exposed to white light, the p-values demonstrate that this trend is not statistically significant the way it is for high-energy light.

The p-values in Table 5 are consistent with the prediction that different light types cause distinct degrees of degeneration. The p-values for the comparison of the three different light types were statistically significant across each pigment, sepia, red, and white, with p-values of 0.00014, 0.00090, and 0.00005 respectively. These results suggest that blue light causes the most severe degeneration, followed by UV light and white light respectively.

Discussion

Addressing Potential Errors and Assumptions

As with all research, it is important to consider potential errors or assumptions in this study. One such possibility is that that there could have been a difference in the image settings that might have caused FLEYE to locate maxima inaccurately, as this function is reliant on the bright spots in the images and could be affected by discrepancies in the image settings. However, this assumption was minimized by ensuring the lighting, background settings, and calibrations were kept constant among all trials and groups, as well as by omitting the images that had unwanted discrepancies (see Sections 2.5, 2.6 and 2.7). There is also a possibility that the light from the LED lamps hit some vials differently than others, causing certain trials to receive an unbalanced level of light exposure; to minimize this error, the position of the lamps and vials were largely left unaltered, and a dark cloth was placed over the setup to prevent extra light from leaving or entering the setup (see Section 2.5).

Connection to Current Research

A recent study observing the impact of blue light on *Drosophila* of different ages validates the assertion that light exposure can induce retinal degeneration in adult fly eyes. The study found that 8 hours of blue light

exposure causes retinal degeneration in 6 day old flies, while 3 hours of blue light exposure increased oxidative stress without causing retinal degeneration. Additionally, the study included a control group in which flies were on a regular schedule of 12 hours white light exposure and 12 hours dark exposure; the control flies had no retinal degeneration and minimal levels of oxidative stress (Hall et. al, 2018). This research is consistent with my findings that blue light is a valid cause of oxidative damage and AMD.

Another study exposed *Drosophila* third instar larvae to blue LED light at various wavelengths for different periods of time. The study found that prolonged exposure could lead to fly death, and mortality increased at shorter wavelengths, specifically between 405 and 466 nanometers. A wavelength of 494 nanometers was found to be ideal for inducing retinal degeneration without causing death, yet exposure to blue light for over 24 hours increased mortality risk. On average, male flies had higher mortality rates than females (Shibuya et. al., 2018). My research used female flies, and in general, there was not a high mortality rate for the flies despite the blue light exposure, which is consistent with the low mortality for female flies found in this study. This research also validates the claim that *Drosophila* larvae could be exposed to blue light to induce retinal degeneration, as done in my project.

These two studies did not test the impact of UV light. My research suggests that along with blue light, UV light is a potential cause for oxidative stress and retinal degeneration, even if it is relatively less severe when compared with the impact of blue light.

Conclusions

Limitations and Future Work

A limitation of this research was that it did not explore any potential treatment options for AMD. Therefore, future work could include the investigation of genes or antioxidant supplementation to alleviate AMD, particularly regarding the white-eyed fly model or blue light exposure method due to the increased susceptibility of these groups to retinal degeneration (based on the conclusions of this study).

Significance and Impact

This research provides insight into the prevalence of high-energy light (blue and UV light) in inducing retinal degeneration, as well as the potential for retinal pigments in suppressing degeneration. Overall, this research improves our currently limited understanding of incurable ocular diseases such as AMD by offering information regarding its pathogenesis and prevention.

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