The Effect of Blue Light on Oxidative Stress in *C. elegans*

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Abstract

Oxidative stress in blue light-exposed *C. elegans* was assessed because adults spend 11 hours per day exposed to excessive blue light, which disrupts circadian rhythm, causes sleep deficit, and accumulation of beta amyloid in the brain. After 24 hours of blue light exposure, *C. elegans* were submerged in dichlorofluorescin diacetate causing the byproducts of oxidative stress to fluoresce. Locomotive ability was measured by counting body bends per second. The oxidative stress in *C. elegans* exposed to blue light (24,827,083 +/- 33,992,523) was significantly greater than those in darkness (986,199 +/- 1,271,535, $t=3.838$, $df=20$, $p<0.05$). Locomotive ability of blue light exposed *C. elegans* (0.400 body bends per second +/- 0.119) was significantly less than those in darkness (0.788 body bends per second +/- 0.199, $t=9.165$, $df=29$, $p<0.05$). Therefore, blue light exposure elevated oxidative stress and reduced locomotive ability.

Introduction

Since the beginning of the electronic age in the 1940's, blue light exposure has increased due to the use of computers, phones, tablets, televisions, and light emitting diodes (LED), (Cohen-Behar et al, 2011). In addition, white light LED lighting has become more common because it is four times more cost efficient than incandescent bulbs. White LEDs are made with a component of blue LEDs because blue light has a lower energy phosphor, which creates solid state light (Itallie, 2014).

Adequate exposure to blue light during daytime hours is necessary, and has been shown to boost energy, alertness, and mood in humans (Chang et al, 2015). However, excessive exposure to blue light, especially in the nighttime hours, can lead to a disrupted circadian rhythm (Breus, 2017), because photosensitive retinal ganglion cells inhibit the production of melatonin, which regulates the body's light and dark cycle (Pei-Ling et al, 2018). Sleep is required to maintain homeostasis. Therefore, if the sleep cycle is disrupted, health problems may result and normal activity may become impaired During sleep in humans, cerebrospinal fluid passes through spaces in the brain to clear away the build up of harmful proteins and toxins, such as beta amyloid, created during daytime activities. During sleep deprivation, the harmful buildup of toxins can lead to Alzheimer's disease (Hamilton, 2013).

Oxidative stress is caused by disruptions in the normal redox processes of cells which leads to an imbalance between antioxidants and free radicals. The resulting production of peroxides and free radicals can damage cell parts, cause DNA base damage and strand breakage, and disrupt metabolic processes (Fawzia et al, 2017).

The average American spends 24h per week looking at a screen and consequently may experience health problems stemming from blue light exposure (Behar-Cohen et al, 2011). Therefore, *C. elegans,* which serve as a model organism, were exposed to 450 nm light for numerous 24 hour sessions to assess the effect of blue light on oxidative stress and locomotive ability (Fawzia et al, 2017).

Materials and Methods

C. elegans age synchronization

C. elegans were subcultured a week prior and allowed to age for four days before the synchronization. The plate was washed with M9 buffer and the worms were transferred to centrifuge tubes (1 ml per tube). After being centrifuged for one minute, the supernatant was removed. 200 µl of distilled water and 800 µl of bleaching solution, composed of 6.25 ml of 4M NaOH, 2.5ml of NaOCl, and 50 ml of M9 buffer, were added to each tube. The *C. elegans* were left for 10 minutes, then checked every thirty seconds until about 90% of the worms had lysed. Before and after washing the *C. elegans* with one ml of M9, the tubes were centrifuged followed by the removal of the supernatant. Two ml of M9 were added to the embryos in the tube before incubating overnight at 20 °C. In a ventilation hood, *C. elegans* were transferred to a petri dish and put in an incufridge for three days. The *C. elegans* were synchronized to ensure that they did not die due to age rather than due to exposure to blue light or the dark.

Exposure of C. elegans to blue light

The synchronized plate of *C. elegans* was either placed in a dark drawer for 24 hours (control), or placed under a 450nm blue light for 24 hours (experimental).

Measurement of oxidative stress

A 50mM dichlorofluorescein diacetate (DCF) stock solution was made by combining 0.0244 grams of DCF and one ml of cell culture grade DMSO. One hour before the application of the DCF, the 50mM stock was diluted 1,000 fold with M9 buffer by serial dilution, and the working solution was vortexed to ensure homogenous concentration. After 23 hours, the *C. elegans* were washed off the petri dish with M9 buffer and transferred into well plates. 50 µl of *C. elegans*, 50µl of M9, and 100 µl of DCF were applied to each well. Within three hours the DCF bonded to the byproducts of oxidative stress formation to cause fluorescence. The greater the intensity of fluorescence, the greater the stress. The wells were viewed under the Zeiss fluorescence microscope and photographs were taken of the *C. elegans* (Figure 1).

Figure 1 Zeiss fluorescence microscope used to view and photograph oxidative stress in *C. elegans* (student produced).

ImageJ software was used to determine the corrected total cell fluorescence (CTCF) in each image using the formula CTCF = (integrated density of *C. elegan)* -(area of *C. elegan)*(mean fluorescence of background). The resulting averages for each group were used to compare stress levels between blue light exposed and unexposed worms.

Locomotive assessment

A plate of *C. elegans* was subcultured one week prior to age synchronization and exposed to either blue light or darkness for 24 hours. The movement of the *C. elegans* was recorded under white light with the zeiss microscope for one minute.

Average body bends per second were calculated from a slow motion version of the one minute video to determine the effects that oxidative stress had on the locomotive ability of *C. elegans*.

Results

Oxidative stress

The blue light exposed group exhibited 92.5% higher oxidative stress compared to that of the control group kept in the darkness (Figure 2). The average corrected total cell fluorescence of the blue light group was 24,827,083 and 1,871,137 in the control group. There was more variability in the oxidative stress group with a standard deviation of 33,992,523 compared to 1,580,165 in the control group. Greater oxidative stress is indicated by more intense fluorescence (Figure 3).

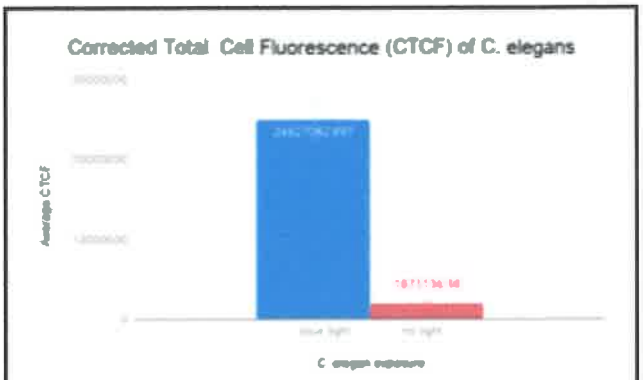

Figure 2 ImageJ was used to determine that the corrected total cell fluorescence (CTCF) was 92.5% greater in blue light-exposed *C. elegans*, than in the control group indicating greater oxidative stress.

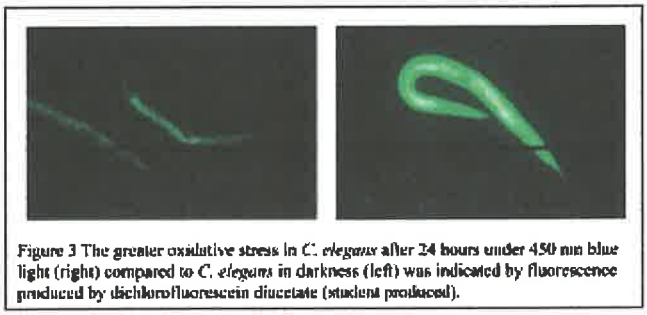

Figure 3 The greater oxidative stress in *C. elegans* after 24 hours under 450 nm blue light (right) compared to *C. elegans* in darkness (left) was indicated by fluorescence produced by dichlorofluorescein diacetate (student produced).

Locomotive ability

C. elegans in the blue light group had impaired locomotive ability compared to the control group.

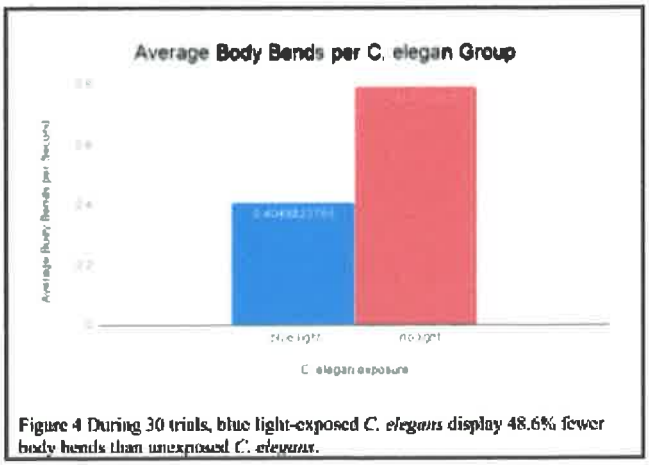

Figure 4 During 30 trials, blue light-exposed *C. elegans* display 48.6% fewer body bends than unexposed *C. elegans*.

Blue light exposed wormed moved at an average of 0.4 body bends per second and the control group worms moved at an average of 0.788 body bends per second (Figure 4). Similar to the oxidative stress trials there was more variability in the blue light groups with a standard deviation of 0.199 compared to that of the control group, 0.119.

Discussion

Blue light exposure resulted in elevated oxidative stress and diminished locomotive ability. *C. elegans* exposed to blue light had an average oxidative stress level (24,827,083 +/- 33,992,523) that was significantly greater than *C. elegans* kept in the dark (1,871,137 +/- 1,580,165, $t=3.695$, $df=29$, $p<0.05$). This may be due to an imbalance caused by the greater production of free radicals than antioxidants in response to the presence of blue light (Betteridge, 2000). Blue light exposed *C. elegans* had a locomotive ability (0.4 body bends per second +/- 0.119) that was significantly less than *C. elegans* kept in the dark (0.788 body bends per second +/- 0.199, $t=9.165$, $df=29$, $p<0.05$). Free radicals may have caused damage to cell structures which takes away from the ability to move because the *C. elegan* was diverting energy to neutralize the excessive free radicals and repair internal structures. Blue light has a high frequency which makes it more difficult for an organism to repair itself than if it was exposed to light with a longer wavelength (De Magalhaes Filho, 2018).

To strengthen the validity of the experiment, the damaging effect of oxidative stress on *C. elegans* could be assessed by identifying the disrupted loci in DNA and RNA.

Also, the ratio of free radicals to antioxidants, before and after exposure to blue light ought to be identified to determine if the blue light led to an increased number of radicals and/or if it caused impairment to the process that neutralizes the radicals, making recovery slower than usual.

Further research would be useful in determining the effects of blue light on *Streptococcus mutans.* Blue light may be able to safely eliminate the bacteria during dental procedures to prevent infection. Also, the ability of epigallocatechin gallate (EGCG), found in green tea leaves, to prevent oxidative stress in blue light exposed *C. elegans,* and to heal *C. elegans* that have already been exposed to blue light could be investigated, this would be the first step in determining the use of EGCG in humans which may lead to a healthier population.

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