Final Report for NASA COOPERATIVE AGREEMENT

#NCC2-779

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T. R / IN / 51

December 31, 1999   *2000 043 738*

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Light-Emitting Diodes and Cool White Fluorescent Light Similarly Suppress Pineal Gland Melatonin and Maintain Retinal Function and Morphology in the Rat

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ABSTRACT

Currently, the light sources most commonly used in animal habitat lighting are cool white fluorescent or incandescent lamps. We evaluated a novel light-emitting diode (LED) light source for use in animal habitat lighting by comparing its effectiveness to cool white fluorescent light (CWF) in suppressing pineal gland melatonin and maintaining normal retinal physiology and morphology in the rat. Results of pineal melatonin suppression experiments showed equal suppression of pineal melatonin concentrations for LED light and CWF light at five different light illuminances (100, 40, 10, 1 and 0.1 lux). There were no significant differences in melatonin suppression between LED and CWF light when compared to unexposed controls. Retinal physiology was evaluated using electroretinography. Results show no differences in a-wave implicit times and amplitudes or b-wave implicit times and amplitudes between 100-lux LED-exposed rats and 100-lux CWF-exposed rats. Results of retinal histology assessment show no differences in retinal thickness rod outer segment length and number of rod nuclei between rats exposed to 100-lux LED and 100-lux CWF for 14 days. Furthermore, the retinal pigmented epithelium and rod outer segments of all eyes observed were in good condition and of normal thickness. This study indicates that LED light does not cause retinal damage and can suppress pineal melatonin at similar intensities as a conventional CWF light source. These data suggest that LED light sources may be suitable replacements for conventional light sources used in the lighting of rodent vivariums while providing many mechanical and economical advantages.

INTRODUCTION

Currently, the light source most commonly used in animal vivariums is broad-band fluorescent white light, sometimes referred to as cool white fluorescent (CWF) light. CWF light and incandescent lights have certain disadvantages over candidate alternative light sources. These include relatively low efficiency (low illuminance/watt of power), higher heat production, and shorter operating life (1), all significant factors when considering operating costs of an animal facility or certain research applications. In addition, they have certain mechanical limitations such as their size and mass. Light emitting diode (LED) technology offers a relatively inexpensive alternative light source and has inherent advantages including: spectral control, high energy efficiency, long operating life, low heat production, ruggedness (solid state), and certain mechanical/size advantages (1).

The purpose of this study was to compare the effectiveness of LED light to CWF light in maintaining normal animal health and well being. We measured light-induced pineal melatonin suppression as an indicator of neuroendocrine function since this is generally accepted as a sensitive measure of the effects of light on the circadian system of rodents. Since light can be phototoxic to the retina (2,3,4) we compared the effects of LED light to CWF light on a) retinal function using light-induced changes in the electroretinogram (ERG), and b) retinal morphology using light microscopy. In a parallel study, we compared LED light to CWF light in maintaining normal behavioral circadian rhythmicity (5).

The profound effect of light on circadian physiology is well established (6) as is the phototoxic effect of light on the retina under certain conditions (2.3.4). However, the 1985 edition of the Guide for the Care and Use of Laboratory Animals states, "Precise lighting

1 requirements for maintenance of good health and physiological stability of animals are not

2 known" (7). Needing exact engineering specifications for animal habitat construction for

3 microgravity studies. NASA sponsored a working group who recommended relatively precise

4 intensity and spectral power specifications for husbandry of rodents and non-human primates (8).

5 The 1996 edition of the Guide for the Care and Use of Laboratory Animals indicated that

6 intensity, duration of exposure. and wavelength are among the factors that should be considered

7 when appropriate illumination is being considered for animal holding room lighting (9). The

8 Guide specifies intensity standards for the albino rat, but does not specify standards for

9 photoperiod or wavelength. The Guide also warns about albino rats' vulnerability to elevated

10 light intensity-induced retinal phototoxicity. We believe that our data adds significantly to the

11 understanding of light intensity on the health and well being of the albino laboratory rat and

12 indicates that an alternative. more economical and efficient lighting system (e.g., LED) can be

13 used for animal husbandry.

14 ## MATERIALS AND METHODS

15 ### Light Sources and Measurement

16 Most commercially available LEDs are individual bulbs that emit restricted bandwidths

17 of light that give distinct color appearances. Groups of monochromatic LEDs, however, can be

18 arranged so as to approximately match the spectral power distribution of CWF light. The LED

19 light source used in these experiments consisted of circular (8-inch diameter), honeycomb arrays

20 of blue, green. yellow and red LEDs (blue LED, catalog # C470-5C18, Cree Research Inc.,

21 Durham, NC; green LED, catalog # HLMP-3950, Future Active Industrial Electronics, San Jose,

22 CA; yellow LED, catalog # HLMP-3850, and red LED, catalog # HLMP-3750,

1   Hamilton/Halmark, Tempe, AZ). These were arranged and powered to give approximately the

2   same spectral power distribution as CWF light. Spectral power distributions were measured

3   using a calibrated radiometer (Model IL-1700, International Light Inc., Newburyport, MA) with a

4   scanning spectral radiometer attachment (Model IL-780/SHD033, International Light Inc.,

5   Newburyport, MA). Figure 1 shows the spectral power distribution curves for the 100-lux CWF

6   and LED light sources used in this study. Currently, when discussing light intensity in human or

7   animal environments, the term "luminous flux" (known as lux) is used, which refers to the

8   amount of light falling on a surface that stimulates the human eye during the daytime. Lux is the

9   standard unit used for specifying lighting for buildings, including buildings which house animals

10 (9). Given this standard, the light stimuli in the following study are characterized in terms of lux.

11   In the melatonin suppression study, illuminances at animals' eye level were measured

12 with a Minolta illuminance meter (Model T-1, Osaka, Japan). Once illuminance was set it was

13 measured with a J16 radiometer and a remote J6512 probe (Tektronix, Inc., Beaverton, OR). The

14 CWF experimental light was produced by an 8-watt CWF bulb (catalog # F8T5, Philips Lighting,

15 Kanagawa, Japan). The LED experimental light array used in this experiment was modified from

16 the original 8-inch diameter, circular, honeycomb array to a more condensed 6-inch diameter

17 circular pattern. The tighter packing of LEDs helped increase the overall maximum illuminance

18 of the LED array. Light from the LED array was also concentrated by use of a fresnel lens

19 (Model A32681, Edmund Scientific Company, Barrington, NJ) in all exposures. Glass neutral

20 density filters (Oriel Corp., Stratford, CT) were used to adjust the intensity of both experimental

21 lights. The duration of the light pulse was automatically controlled by a variable timer (Solar

22 Light Co., Philadelphia, PA) attached to an iris diaphragm.

1   In the retinal phototoxicity study. illuminances at animals' eye level were measured using

2   a calibrated radiometer (Model IL-1700. International Light Inc.. Newburyport, MA) with a

3   photometer sensor (Model # SED038, Serial # 2064 using Y filter # 8990 and W diffuser # 4945,

4   International Light Inc., Newburyport, MA). Direct energy was measured (in $\mu W/cm^2$) with the

5   same calibrated radiometer and an irradiance sensor (Model # SED038, Serial # 2143 using F

6   filter #18629 and W diffuser #9089, International Light Inc., Newburyport, MA). The range of

7   illuminances produced by the photic stimulus during the electroretinographic assessment was

8   measured using the same calibrated radiometer and the photometer sensor.

9   **Melatonin Suppression Study**

10   All experiments performed in this study were reviewed and approved by Jefferson

11   Medical College Institutional Animal Care and Use Committee and San Jose State University

12   Institutional Animal Care and Use Committee to assure that experiments minimized any potential

13   pain and discomfort of experimental animals. Adult male Sprague Dawley rats (175-200g,

14   Harlan Sprague Dawley Breeding Laboratories. Indianapolis, IN) were maintained on a 12:12

15   light:dark (LD) cycle (lights on 0600-1800 h) for a minimum of 2 weeks before experimentation.

16   The daily light cycle was produced by 40-watt CWF light sources (catalog # F40-CW, Sylvania,

17   Danvers, MA). which provided an illuminance of 60 ± 6 lux (18 ± 2 $\mu W/cm^2$) at the animal's eye

18   level. Animal handling was carried out under dim red light produced by a 25-watt incandescent

19   bulb behind a Kodak 1A safelight filter (Eastman Kodak, Rochester, NY). The red light had an

20   illuminance of 0.4 - 1.5 lux.

21   Each of the melatonin suppression experiments (five total: each representing a different

22   exposure intensity) took place between 0000 h and 0230 h on separate days. Each exposure

5

1   included 3 groups of eight rats: a group exposed to CWF light, a group exposed to the LED array

2   and an unexposed control group. Therefore, using 5 different illuminances, 3 treatment groups

3   and 8 animals per treatment group, a total of 120 animals were used in the melatonin suppression

4   study. Each exposure lasted 5 min and occurred while the rats were dark-adapted. Five different

5   light illuminances were tested: 100 lux, 40 lux, 10 lux, 1.0 lux, and 0.1 lux which correspond to

6   the following LED and CWF irradiances, respectively: 32.0 $\mu W/cm^2$ and 34.8 $\mu W/cm^2$, 11.5

7   $\mu W/cm^2$ and 13.0 $\mu W/cm^2$, 3.40 $\mu W/cm^2$ and 3.23 $\mu W/cm^2$, 0.30 $\mu W/cm^2$ and 0.36 $\mu W/cm^2$,

8   0.036 $\mu W/cm^2$ and 0.044 $\mu W/cm^2$. Rats were individually exposed to their respective light

9   source (LED or CWF) for 5 min in a 18.5 x 10.5 x 12.5 cm flat black box. The exposure system

10 was a modification of an apparatus described elsewhere (10,11). Following the 5-min light

11 exposure, rats were transferred to a light-tight holding box where they were held in darkness for

12 15 min. Rats were then euthanized by decapitation and pineal glands were removed, placed in a

13 microtiter plate and frozen at -20 °C.

14   The pineal glands were later assayed for melatonin content using a modification of the

15 radioimmunoassay (RIA) described by Rollag and Niswender (12). In this procedure, pineal

16 glands were suspended in 200 μL PBS-0.1% gelatin and dispersed with a microultrasonic cell

17 disrupter (Kontes, Vineland, NJ). Duplicate 20-μL samples of the sonicant were each placed into

18 an additional 180 μL PBS-0.1% gelatin. Each duplicate (200 μL volume) was then incubated

19 with 100 μL of [^{125}I] (40,000cpm/100 μL or 57,000dpm/100 μL) melatonin analog and 100 μL of

20 a 1:64,000 dilution of rabbit antiserum (antibody raised by Mark D. Rollag on 9/16/74 in rabbit #

21 R1055) to give a final volume of 400 μL. After a 2-day incubation at 4°C, antibody-bound

22 radioactivity was precipitated by adding 3mL cold (4°C) 96% ethanol to the incubation mixture

6

1 and centrifuging at 1000 x g for 30 min. The intra- and interassay coefficients of variation were

2 less than 10% each. Each melatonin assay included an unexposed control group, an LED-

3 exposed group and a CWF-exposed group from one light illuminance cohort.

4   For each study (each illuminance), all data from the CWF-exposed, LED-exposed and

5 control animals were compared by analysis of variance (Kruskal-Wallis one-way ANOVA used

6 because variances between groups were unequal), where differences between groups were

7 considered significant if $p \leq 0.05$. Significant differences were further analyzed using the

8 Student-Newman-Keuls Method for multiple comparisons with an experiment-wise alpha of 0.05

9 for each illuminance.

10 **Retinal Phototoxicity Study**

11   All experiments performed in this study were reviewed and approved by the San Jose

12 State University Institutional Animal Care and Use Committee to assure that experiments

13 minimized any potential pain and discomfort of experimental animals. In the retinal

14 phototoxicity study, the animals were housed under either LED lighting or CWF lighting.

15 Sixteen male Sprague Dawley rats (300-350g, Simonsen Laboratories, Gilroy, CA) were used in

16 this experiment and were housed and tested together in groups of four: two control animals

17 (CWF-exposed rats) and two experimental animals (LED-exposed rats). CWF-exposed rats were

18 housed in a chamber fitted with two 14-watt CWF bulbs (catalog # GE F14T12, GE Lighting,

19 Cleveland, OH). LED-exposed rats were housed in a separate chamber fitted with two LED

20 arrays. Individual rats were kept in shoebox-type cages fitted with aluminum sheet metal shaped

21 in a cylindrical ring (8 inches in diameter and 8 inches in height). This design helped provide

22 uniform light exposure to the animal as well as an unobstructed light path. The light sources

7

1   were mounted from the ceilings of each of the chambers, to ensure that each animal would be

2   exposed directly from overhead with one CWF bulb or LED panel. The desired CWF irradiance

3   was produced by masking the CWF bulbs with aluminum foil as well as adjusting the distance

4   between the CWF bulbs and the rat cages. The desired LED irradiance was established by

5   adjusting the distance between the LED panel and the rat cages. The illuminance used in this

6   study was 100 lux (corresponding irradiances were 28.5 μW/cm^2 from the LED panel and 30.1

7   μW/cm^2 from the CWF source). The two exposure chambers, one exposing two rats to CWF

8   fluorescent light and the other exposing two rats to LED light, were both constructed and

9   maintained so as to keep external light from entering.

10   Rats were exposed to their respective light source using 12:12 LD cycles for 14 days,

11   with lights on at 0700 hours. Both the duration of exposure and the cyclic lighting schedule are

12   important factors when determining the extent of light-induced retinal damage (13). Quantitative

13   changes in retinal function and morphology following toxic light exposure are most reliably

14   measured on the 14th day or beyond the start of toxic light exposure (14). All animal handling

15   and maintenance was done under dim red lighting (Kodak Safelight #2 - Eastman Kodak,

16   Rochester, NY) which had an illuminance of 0.4 - 1.5 lux.

17   **Electroretinography**

18   On the fifteenth day of the retinal phototoxicity study, rats were moved to a darkroom

19   following dark adaptation overnight (the last dark cycle of the 14th day) for scotopic

20   electroretinography (ERG) assessment. Ninety five percent of the rat retina is comprised of rod

21   photoreceptor cells that are best studied in dark adaptation (15). ERG was performed on the rats

1   in the morning no earlier than 0930 hours to avoid variability associated with photoreceptor disc

2   shedding (16).

3   The rats were injected i.p. with urethane (0.15g/kg body weight; 20% aqueous solution).

4   Pupils were dilated with 10% phenylephrine eye drops. After anesthesia, the rat's eyelid was

5   held open and the cornea was protected from drying with methylcellulose drops. A

6   photostimulator producing white light (Grass PS22 Photo Stimulator, Grass Medical Instruments,

7   Quincy, MA) was used as a stimulus control to confirm an ERG response in the rats. Monocular

8   recordings were obtained using a 3mm diameter loop of platinum-iridium wire as the recording

9   electrode on the cornea, a reference electrode on the forehead and a ground electrode on the

10   cheek. The signals were AC-amplified with 0.1-kHz (low frequency) and 1-kHz (high

11   frequency) cutoffs and displayed on a PC monitor for cursor placement and amplitude

12   measurement, using the computerized BPM-100 ERG recording system (RetinoGraphics, Inc.,

13   Norwalk, CT). The photic stimulus used in the ERG recordings was a blue LED light placed a

14   standard distance of 9 cm above the eye to illuminate the entire retina. This methodology was

15   standardized across individuals, with elicited ERG waveforms confirmed with the Grass PS22

16   Photo Stimulator control. ERG responses to a series of brief flashes varying in intensity were

17   recorded, starting at the highest intensity and stepping down to the lowest intensity. Each step in

18   intensity represented a 0.5-log unit. The range of illuminance of the brief flashes was from a

19   maximal 130 lux to 0.1 lux. The range of light intensity was controlled by the BPM-100 ERG

20   recording system which adjusts the power of the stimulus electrically rather than by placement of

21   neutral density filters in the path of the light.

1   The average of eight responses was obtained at each intensity, with an interstimulus

2   interval of 20 seconds. Later the ERG waveforms were measured using cursor placement to

3   determine the a- and b-wave amplitudes and implicit times. These values were plotted against

4   the stimulus intensity (intensity-response functions). Statistical evaluation of the data used two

5   way-ANOVA with ERG stimulus as a within-subjects factor and light source as a between-

6   subjects factor, where results were considered significant if $p \leq 0.05$. Stimulus intensity was

7   confirmed by direct energy measurement, at the beginning of the experiment and at the end of the

8   experiment. Upon completion of ERG data collection, each rat was sacrificed with CO_2 gas. The

9   right eyes were removed and placed in modified Karnovsky's fixative for histological preparation

10   and analysis.

11   **Retinal Histology**

12   To determine whether light damage occurred in the retinas of the LED and CWF-exposed

13   rats of the retinal phototoxicity study, the retinal epithelium, rod outer segments, rod nuclei, and

14   total retinal thickness were inspected. The quantitative histological analysis was carried out on 7

15   LED-housed rats from the ERG study, 7 of the CWF-housed rats from the ERG study, as well as

16   3 control rats that were not subjected to the ERG procedure. The 3 control rats were not exposed

17   to the experimental chamber environments, but maintained on a 12:12 LD cycle of CWF light

18   (250 lux, lights on at 0700) in an animal vivarium for 14 days. Eyes were fixed by immersion in

19   modified Karnovsky's fixative (1% paraformalyde, 1.5% glutaraldehyde in cacodylate buffer) for

20   at least 72 hours. Following fixation, each eye was postfixed in 2% osmium tetroxide, bisected

21   on the vertical meridian and embedded in epon-araldite plastic.

1   The retinal tissue was sectioned with a Sorval JB-4 ultramicrotome (Dupont

2   Instruments). Semi-thin sections were stained with toluidine blue (1% in 1% borax) and were

3   examined on a Nikon research microscope with an ocular micrometer attachment. Retinal

4   measurements were made under 100x oil immersion at 270 microns and 450 microns from the

5   optic nerve head.

6   The retinal pigmented epithelium was inspected for the presence of missing areas of

7   retinal pigmented epithelium, vacuolation and vesicles. The rod outer segment length was

8   measured and observed for any signs of damage and disorganization. The number of rod nuclei

9   was counted perpendicular to the retinal pigmented epithelium. Lastly, the thickness of the retina

10 was measured perpendicular to the retinal pigmented epithelium. Statistical evaluation of the data

11 used one-way ANOVA, where differences between groups were considered significant if $p \leq$

12 0.05.

13 **RESULTS**
14
15 **Melatonin Suppression Study**

16 Figure 2 shows the pineal melatonin concentrations for each of the 3 groups of rats at

17 their respective light exposure intensities. Because each light exposure experiment took place on

18 different days (often seperated by weeks), animals were compared to their respective control

19 groups. In both experimental light conditions (CWF and LED), at all illuminances examined

20 (100, 40, 10, 1, and 0.1 lux), the rats showed significant melatonin suppression ($p \leq 0.05$ for

21 control vs. CWF and control vs. LED; Table 1). In no case was the melatonin suppression

22 induced by an LED illuminance significantly different from the melatonin suppression elicited by

23 the same illuminance of CWF light ($p > 0.05$ for LED vs. CWF at all illuminances).

1   For the 100-lux intensity experiment, the control, CWF, and LED groups showed pineal

2   melatonin concentrations of 1166 ± 136, 393 ± 41, and 439 ± 25 pg/gland (mean ± SEM),

3   respectively. For the 40-lux intensity experiment, the control, CWF, and LED groups showed

4   pineal melatonin concentrations of 1569 ± 126, 365 ± 34, and 462 ± 50 pg/gland (mean ± SEM),

5   respectively. For the 10-lux intensity experiment, the control, CWF, and LED groups showed

6   pineal melatonin concentrations of 353 ± 34, 257 ± 13, and 231 ± 6 pg/gland (mean ± SEM),

7   respectively. For the 1.0-lux intensity experiment, the control, CWF, and LED groups showed

8   pineal melatonin concentrations of 650 ± 124, 218 ± 42, and 164 ± 12 pg/gland (mean ± SEM),

9   respectively. For the 0.1-lux intensity experiment, the control, CWF, and LED groups showed

10   pineal melatonin concentrations of 464 ± 85, 239 ± 71, and 158 ± 12 pg/gland (mean ± SEM),

11   respectively.

12   **Retinal Phototoxicity Study**

13   No evidence of retinal phototoxicity was found in the ERG responses from rats housed

14   in LED or CWF light. Examples of individual ERG waveforms are shown in Figure 3. ERG

15   response-intensity curves are shown (mean ± 1 S.D.) for both exposure groups in Figures 4 - 7.

16   Figures 4 and 5 plot the a-wave implicit times and amplitudes for the LED and CWF groups.

17   Figures 6 and 7 plot the b-wave implicit times and amplitudes for the LED and CWF groups.

18   ERG a-waves were rarely observed in the lower photic stimulus intensities and were undetectable

19   below the -1.5 log intensity in both LED and CWF animals. The mean implicit times and

20   amplitudes of the ERG a- and b-waves were not significantly different for the two

21   treatment/exposure groups (Table 2: Light Source). ERG stimulus intensity affected a-wave and

22   b-wave implicit times and amplitudes as expected (Table 2: ERG Stimulus). Lastly, there was no

1 interaction between light source and ERG stimulus for any of the four ERG measurements (Table

2 2: Light source x ERG Stimulus).

3 **Retinal Histology**

4 No evidence of phototoxicity was found in retinal morphology. Table 3 shows the

5 results of the quantitative measurements performed on the rat retinas. The retinas, including the

6 retinal pigmented epithelium and rod outer segments, of all the eyes were in good condition and

7 their thickness was normal. No significant differences were found between LED, CWF and

8 control groups in the measurements of rod outer segment length. number of outer nuclear layer

9 nuclei. or overall thickness of the retina (Table 3, one-way ANOVA).

10 # DISCUSSION

11 This study shows that a novel LED light source can suppress pineal gland melatonin

12 concentrations equivalent to a conventional CWF light source at similar intensity levels. Also,

13 we have demonstrated that there are no significant differences in retinal function (determined by

14 ERG) or retinal structure (determined by light microscopy) for animals exposed to LEDs

15 compared to animals exposed to CWF lighting. In addition, our parallel study as reported in (5),

16 shows LED light to maintain normal circadian entrainment as assessed through behavioral

17 parameters such as gross locomotor activity, drinking and feeding. To compare our results to

18 national standards for specifying light in animal housing facilities (9). we have framed the studies

19 reported here in terms of photometry. We have reported our light intensities in radiometric terms

20 ($\mu W/cm^2$) for greater utility to the research community.

1   Light suppression of pineal gland melatonin was used as a bioassay in this study because

2   it is a sensitive measure of the effects of light on the circadian system of rodents and other

3   species. The sensitive nature of the retinal-hypothalamic-pineal axis in the white laboratory rat

4   was initially demonstrated by Minneman and colleagues (17). The exposure of the mammalian

5   retina to light during the night induces neuronal signals in the retinohypothalamic pathway,

6   which in turn elevates glucose metabolism in the suprachiasmatic nuclei of the hypothalamus

7   (18). Signals from the suprachiasmatic nuclei are transmitted to the pineal gland which leads to

8   rapid suppression of pineal enzymes and subsequent lowering of pineal gland melatonin (19).

9   The suprachiasmatic nuclei have been known to be the endogenous pacemakers for circadian

10   rhythms in mammals, including the rhythmic pineal gland synthesis and secretion of melatonin

11   (20). This response of the retinal-hypothalamic-pineal axis to light is dependent on the intensity

12   (21) and wavelength (22) of the light. Therefore, the ability of each light source to suppress

13   pineal gland melatonin at each of the light intensities used provided a meaningful comparison

14   between LED light and CWF light. The data from these experiments indicate that there is no

15   significant difference in melatonin suppression between rats exposed to CWF light and rats

16   exposed to LED light at illuminances ranging from 100 lux to 0.1 lux. The significant

17   suppression at the lowest illuminance suggests that even lower intensities of CWF and LED light

18   might suppress pineal gland melatonin. The ability of LED light to suppress pineal gland

19   melatonin concentrations significantly at low illuminances, comparable to that of the CWF light,

20   suggests that it can be equally effective in regulating other aspects of circadian system

21   physiology. Further tests on other circadian parameters are required to confirm this.

1   Light exposure can cause retinal damage and irreversible loss of photoreceptor cells,

2   causing partial or total blindness (2,3,4,23,24). It was especially important to determine whether

3   the LED arrays caused phototoxicity because of the narrow wavelength band in component

4   LEDs, as light damage is produced more readily by some wavelengths of visible light (2). To

5   determine whether the use of LED lighting in animal habitats would have any deleterious effect

6   on the retina, we compared effects of LED animal habitat lighting and CWF animal habitat

7   lighting on retinal function using ERG assessment. If there was functional damage to the retina,

8   it would be observed by changes in the electroretinogram. The ERG is the evoked mass response

9   of the retina to a flash of light (25). The amplitudes of the individual components of the

10   composite ERG waveform can be related to specific cellular layers of the neural retina and the

11   retinal epithelium (25). The a-wave is the first part of the ERG and is the component produced

12   by the photoreceptor cells (25). The b-wave, which is the most salient fast potential of the ERG

13   of the intact retina, is a result of changes in extracellular potassium ion concentrations in Muller

14   cells as a result of on-bipolar excitation (25). Loss of retinal function would be indicated by the

15   reduction in amplitude of the ERG a- and b-waves, showing a reduced sensitivity to light or

16   functional loss. In addition to loss of visual function, retinal phototoxicity may also affect the

17   hypothalamic-pineal axis due to the transmission of signals from the eye to the central pacemaker

18   of physiological and behavioral rhythms. The results of the ERG assessment show that LED

19   does not reduce photoreceptor sensitivity or disrupt retinal function (Figures 4 - 7).

20   Morphological assessment was performed following the ERG functional assessment in

21   order to determine the presence of any photoreceptor cell damage or death. Past findings have

22   shown photoreceptor degeneration to occur in albino rats exposed to light intensities greater than

15

1 60 lux for 13 weeks using a 12:12 LD cycle (26). Another study showed no signs of retinal

2 degeneration in rats exposed to 194 lux for 4 - 6 months using 14:10 LD cycle (4). The

3 histological data obtained in our study support the conclusion that 100-lux LED lighting for 14

4 days using a 12:12 LD cycle does not cause retinal damage (Table 3).

5   Investigating the effects of light on mammalian physiology involves the discipline of

6 photobiology, the interaction between optical radiation and living organisms. Specifically,

7 photobiology is the study of how the infrared, visible and ultraviolet portions of the

8 electromagnetic spectrum influence biological processes (27,28). Light, however, is often

9 understood and described relative to the human visual system, where most individuals discuss it

10 in terms of its apparent color and brightness. This description of light is serviceable for purposes

11 of general communication, but is less useful as a descriptor in photobiology. There are two broad

12 categories of light measurement techniques: radiometric and photometric (29). Radiometry is

13 based exclusively on the physical properties of light, such as its energy and wavelength, whereas

14 photometry is based on the selective responsiveness of the human visual system (29,30).

15 Radiometric quantification of stimuli is particularly important in photobiological research. A

16 radiometer measures the radiant power of a light source over a defined range of wavelengths,

17 while a photometer measures the luminous flux falling on a surface that stimulates the human eye

18 during the daytime. A photometer is simply a radiometer that has filters added to the detector

19 which "shape" the detector sensitivity to resemble the luminance (brightness) response of the

20 human visual response of the "standard observer" as determined by the CIE (30). Thus,

21 photometry is a special branch of radiometry. Although the photometric system provides a

22 serviceable nomenclature and measurement technique for describing the light stimuli used in this

1   manuscript. it does not imply that the investigators accept that lux measurements are specifically

2   relevant to circadian and neuroendocrine responses in rats. To the contrary, the photoreceptive

3   physiology in rodents for circadian and neuroendocrine regulation has not been identified (31). It

4   is very unlikely that the visual sensitivity of the human eye will be equivalent to the sensitivity of

5   the input physiology of the rodent circadian system. Once again, we have framed these studies in

6   terms of photometry in order to compare our results to national standards for specifying light in

7   animal housing facilities (9). The optimal measurement for circadian and neuroendocrine

8   regulation in animals will only be resolved when the photobiological physiology which mediates

9   these effects of light is clarified.

10   In conclusion, we found that LED light may be a suitable alternative to CWF light for

11   animal habitat lighting. Replacing incandescent or fluorescent light sources with LEDs carries a

12   number of advantages including longer operating life, less mass and volume, less heat

13   production. less power consumption and higher efficiency (candela/watt of power)(1). In

14   addition to the mechanical and economical advantages, LEDs can be used to produce more

15   precisely timed and spectrally controlled photic stimuli. LED light appears to support normal

16   circadian physiology and caused no functional damage or morphological destruction in the retina.

17   Furthermore, we believe the data reported in this study, using alternate light sources and showing

18   melatonin suppression at low illuminances (0.1 lux) as well as no retinal phototoxicity under

19   higher illuminances (100 lux), contribute significant new information to our understanding of the

20   effects of light on lab animal health and well being.

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Acknowledgments.

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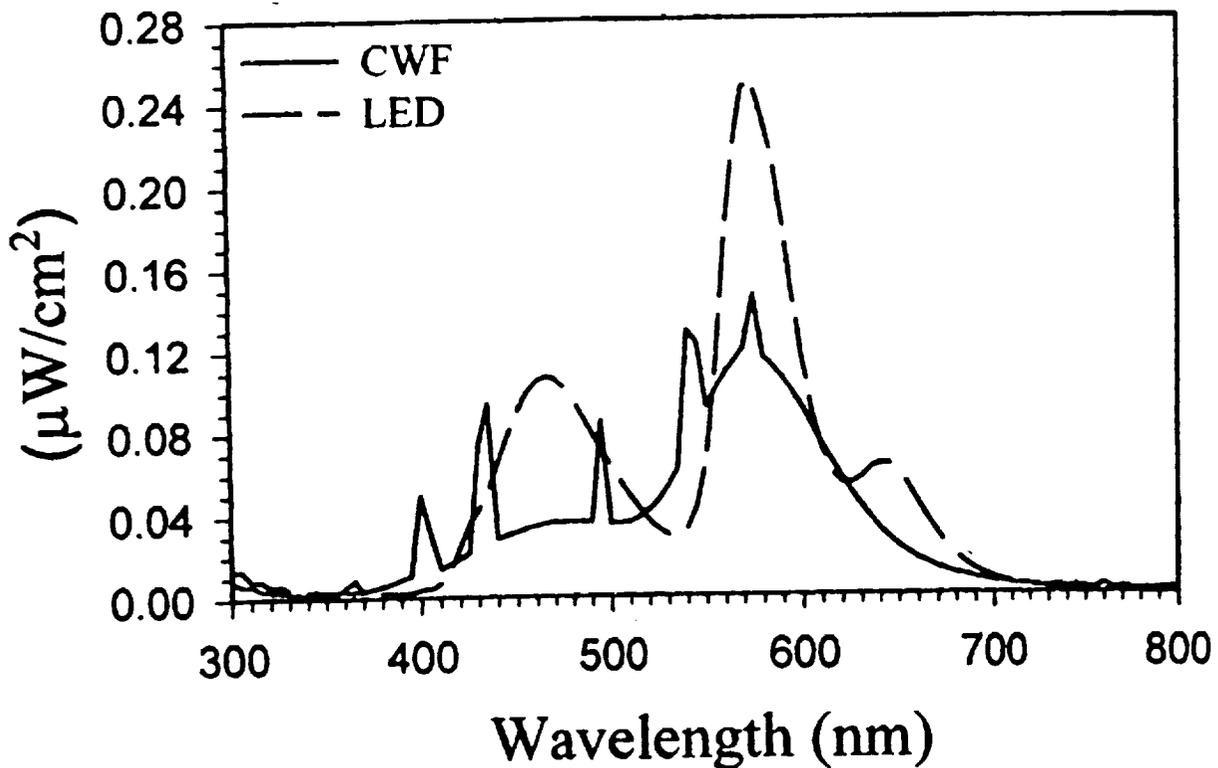
Figure 1. Spectral power distribution curves of LED and CWF light sources used in this study. Energy distribution for LED array is as follows: blue (35.8%), green & yellow (47.2%) and red (17%). Energy distribution for CWF light source is as follows blue (34.6%), green & yellow (48.6%) and red (16.8%). Bandwidths with corresponding wavelength ranges and photon energies are as follows: Blue(460-490nm/2.53-2.70eV), Green(490-575nm/2.16-2.53eV), Yellow(575-585nm/2.12-2.16eV) and Red(760-610nm/1.63-2.03eV).
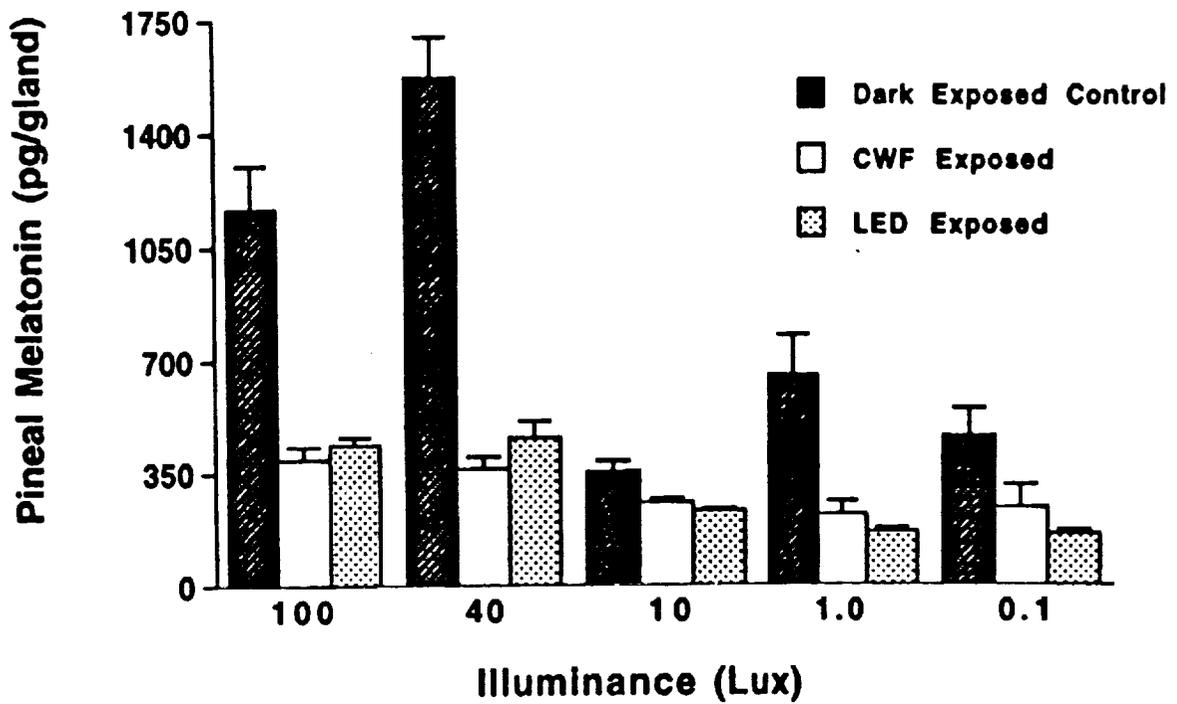
Figure 2. Pineal melatonin suppression in control (dark adapted), CWF-exposed and LED-exposed rats at 5 different light illuminances. Values are mean ± SEM, n=8 per group. Melatonin was significantly reduced by light exposure but there were no differences in melatonin between CWF and LED animals. Abbreviations: Light Emitting Diode (LED), Cool White Fluorescent (CWF).
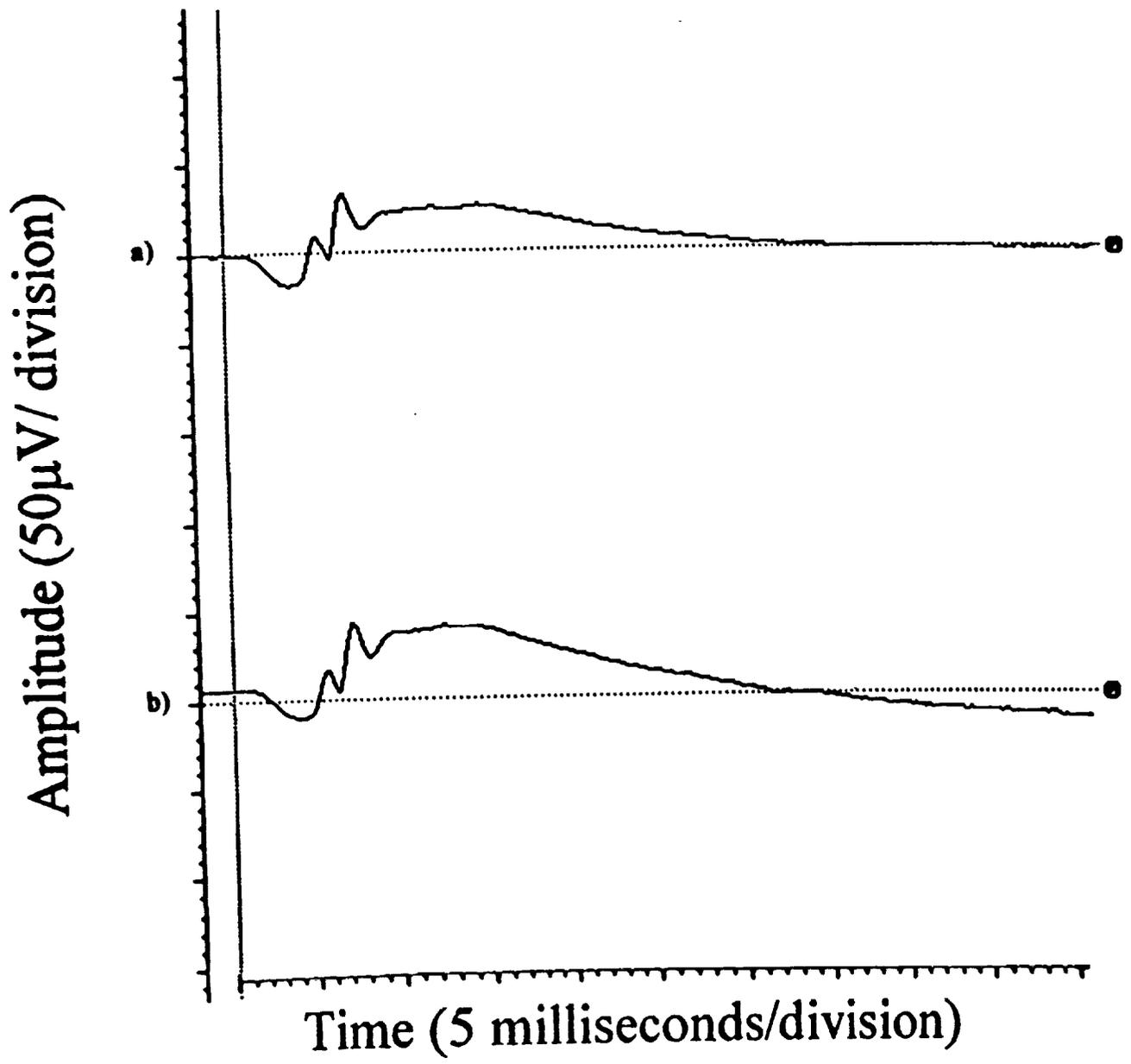
Figure 3. ERG waveforms evoked (obtained with a monochromatic blue stimulus) from a rat housed in CWF light (a) and a rat housed in LED light (b).

Figure 4. ERG A-wave Implicit Times (Mean ± 1 S.D., n=7) from rats housed in LED and CWF light.
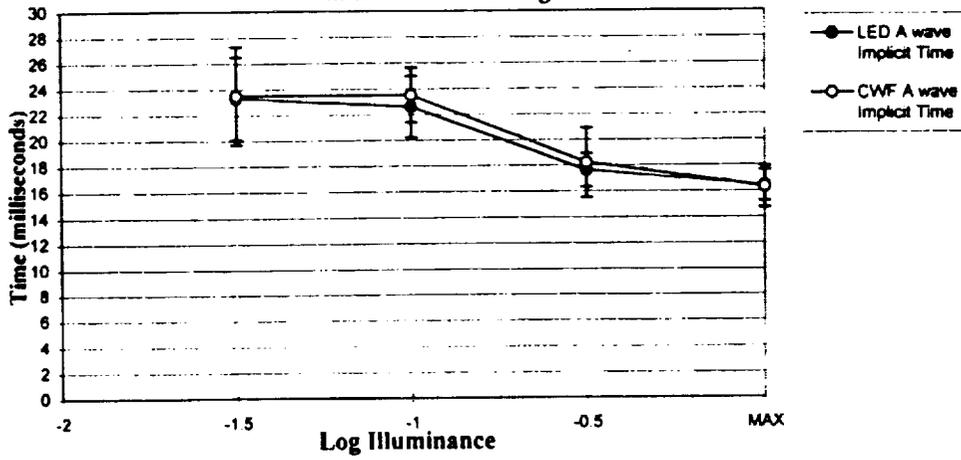
Figure 5. ERG A-wave Amplitudes (Mean ± 1 S.D., n=7) from rats housed in LED and CWF light.
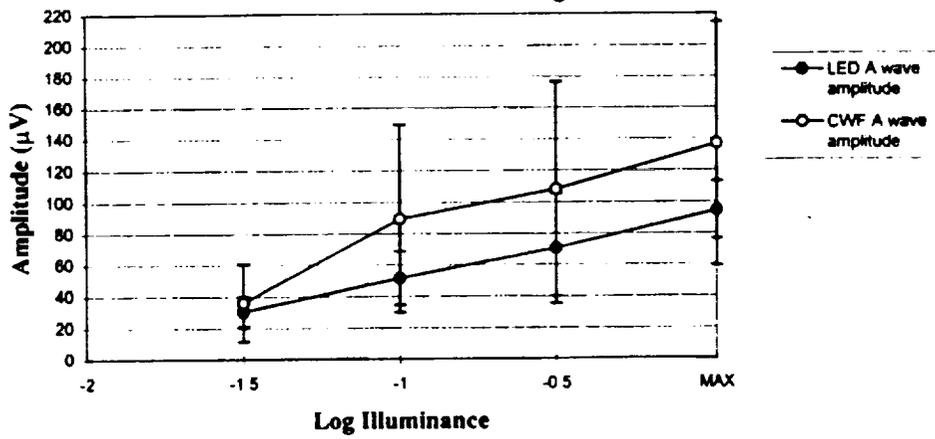
Figure 6. ERG B-wave Implicit Times (mean ± 1 S.D., n=7) from rats housed in LED and CWF light.
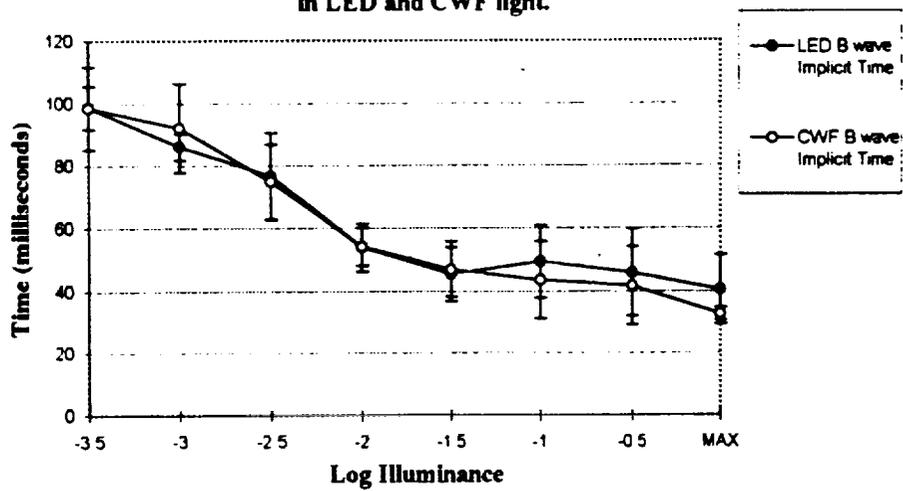
Figure 7. ERG B-wave Amplitudes (Mean ± 1 S.D., n=7) from rats housed in LED and CWF light.
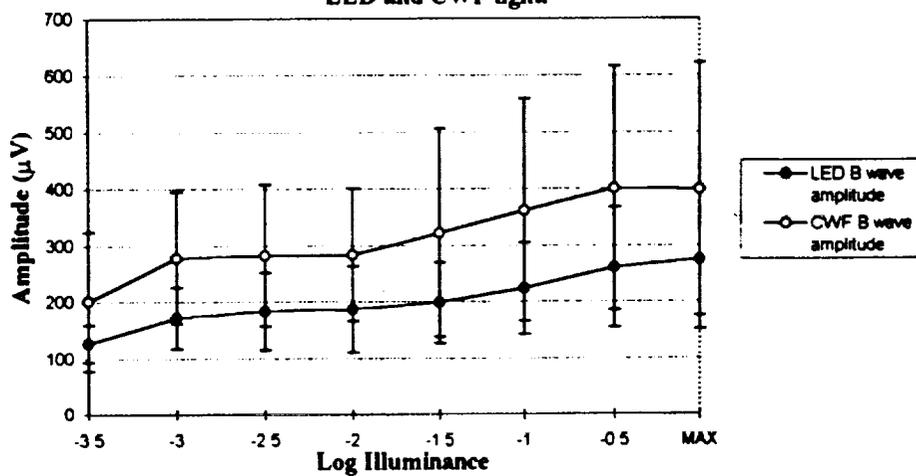
Table 1. Results of Kruskal-Wallis one-way ANOVA for each of the five illuminances studied in the melatonin suppression study. N = 8 per group (3 groups: Control. CWF and LED). P-values for Student-Newman-Keuls multiple comparisons are given in the text.

Illuminance (lux)	Result	p-value
100	H(2) = 15.765	≤ 0.001
40	H(2) = 16.980	≤ 0.001
10	H(2) = 9.219	p = 0.01
1	H(2) = 13.132	p = 0.001
0.1	H(2) = 13.223	p = 0.001

Table 2. Results of the two-way ANOVA's performed on each of the four electroretinogram measurements (a-wave implicit time, a-wave amplitude. b-wave implicit time, and b-wave amplitude) taken during the phototoxicity/ERG assessment study. N = 7 per group (2 groups: CWF and LED). ERG Stimulus was a repeated measures factor in the ANOVA's.

Measurement	Light Source	ERG Stimulus	Light Source x ERG Stimulus
a-wave implicit time	$F_{(1,12)}=0.130$, $p=0.725$	$F_{(3,34)}=60.504$, $p<0.001$	$F_{(3,34)}=0.129$, $p=0.942$
a-wave amplitude	$F_{(1,12)}=1.663$, $p=0.221$	$F_{(3,34)}=26.477$, $p<0.001$	$F_{(3,34)}=0.914$, $p=0.444$
b-wave implicit time	$F_{(1,12)}=0.252$, $p=0.625$	$F_{(7,84)}=83.210$, $p<0.001$	$F_{(7,84)}=0.751$, $p=0.629$
b-wave amplitude	$F_{(1,12)}=3.364$, $p=0.092$	$F_{(7,84)}=9.815$, $p<0.001$	$F_{(7,84)}=0.365$, $p=0.920$

Table 3. Summary of retinal histology (mean ± 1 S.D.) performed on LED-housed rats (n=7), CWF-housed rats (n=7), and Control rats (n=3). One-way ANOVA performed on data with results shown.

	Control	CWF	LED	ANOVA
Rod Outer Segment Length (μm)	24.38 ± 4.51	24.20 ± 6.35	22.14 ± 3.20	$F_{(2,14)} = 0.375$, $p = 0.694$
# Rod Nuclei, outer nuclear layer	9.67 ± 0.80	9.32 ± 0.97	9.00 ± 0.88	$F_{(2,14)} = 0.606$, $p = 0.559$
Retinal Thickness (μm)	188.33 ± 27.88	210.8 ± 42.48	186.07 ± 32.10	$F_{(2,14)} = 0.902$, $p = 0.428$