

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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Background and Purpose: A novel light-emitting diode (LED) light source for use in animal-habitat lighting was evaluated.

Methods: The LED was evaluated by comparing its effectiveness with that of cool white fluorescent light (CWF) in suppressing pineal gland melatonin content and maintaining normal retinal physiology, as evaluated by use of electroretinography (ERG), and morphology.

Results: Pineal melatonin concentration was equally suppressed by LED and CWF light at five light illuminances (100, 40, 10, 1, and 0.1 lux). There were no significant differences in melatonin suppression between LED and CWF light, compared with values for unexposed controls. There were no differences in ERG 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 histologic examination indicated 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, in all eyes, the retinal pigmented epithelium was intact and not vacuolated, whereas rod outer segments were of normal thickness.

Conclusion: LED light does not cause retinal damage and can suppress pineal melatonin content at intensities similar to CWF light intensities.

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. The CWF and incandescent lights have certain disadvantages over candidate alternative light sources. These include low efficiency (low illuminance per 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 size and mass. Light emitting diode (LED) technology offers an 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 and size advantages (1).

The purpose of the study reported here was to compare the effectiveness of LED light with that of CWF light in maintaining normal animal health and well being. We measured light-induced pineal melatonin suppression as an indicator of neuroendocrine function because this is generally

accepted as a sensitive measure of the effects of light on the circadian system of rodents. Because light can be phototoxic to the retina (2–4), we compared the effects of LED light with those of CWF light on: retinal function, using light-induced changes in the electroretinogram (ERG); and retinal morphology, using light microscopy. In a parallel study, we compared LED light with 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 (2–4). However, the 1985 edition of the *Guide for the Care and Use of Laboratory Animals* states, “Precise lighting requirements for maintenance of good health and physiological stability of animals are not known” (7). Needing exact engineering specifications for animal-habitat construction for microgravity studies, the National Aeronautics and Space Administration sponsored a working group who recommended precise intensity and spectral power specifications for husbandry of rodents and nonhuman primates (8). The 1996 edition of the *Guide for the Care and Use of Laboratory Animals* indicated that intensity, duration of exposure, and wavelength are among the factors that should be considered when appropriate illumination is being considered for animal holding-room lighting (9). The Guide specifies intensity standards for the albino

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rat, but does not specify standards for photoperiod or wavelength. The *Guide* also warns about albino rats' vulnerability to increased light intensity-induced retinal phototoxicity. We believe that our data add substantially to the understanding of light intensity on the health and well being of the albino laboratory rat and indicate that an alternative, more economical and efficient lighting system (e.g., LED) can be used for animal husbandry.

Materials and Methods

Light sources and measurement: Most commercially available LEDs are individual bulbs that emit restricted bandwidths of light that give distinct color appearances. Groups of monochromatic LEDs, however, can be arranged so as to approximately match the spectral power distribution of CWF light. The LED light source used in these experiments consisted of circular (8-in.-diameter), honeycomb arrays of blue, green, yellow, and red LEDs (blue LED, catalog no. C470-5C18, Cree Research Inc., Durham, N.C.; green LED, catalog no. HLMP-3950, Future Active Industrial Electronics, San Jose, Calif.; yellow LED, catalog no. HLMP-3850, and red LED, catalog no. HLMP-3750, Hamilton/Halmark, Tempe, Ariz.). These were arranged and powered to yield approximately the same spectral power distribution as that of CWF light. Spectral power distributions were measured, using a calibrated radiometer (model no. IL-1700; International Light Inc., Newburyport, Mass.) with a scanning spectral radiometer attachment (model no. IL-780/SHD033; International Light Inc.). Figure 1 shows the spectral power distribution curves for the 100-lux CWF and LED light sources used in this study. Currently, when discussing light intensity in human or animal environments, the term "luminous flux" (known as lux) is used, which refers to the amount of light falling on a surface that stimulates the human eye during the daytime. Lux is the standard unit used for specifying lighting for buildings, including buildings that house animals (9). Given this standard, the light stimuli in the study reported here are characterized in terms of lux.

In the melatonin suppression study, illuminances at the animal's eye level were measured by use of a Minolta illuminance meter (model no. T-1; Osaka, Japan). Once illuminance was set, it was measured by use of a J16 radiometer and remote J6512 probe (Tektronix, Inc., Beaverton, Oreg.). The CWF experimental light was produced by an 8-watt CWF bulb (catalog no. F8T5; Philips Lighting, Kanagawa, Japan). The LED experimental light array used in this experiment was modified from the original 8-in.-diameter, circular honeycomb array to a more condensed 6-in.-diameter circular pattern. The tighter packing of LEDs helped increase the overall maximal illuminance of the LED array. Light from the LED array was also concentrated by use of a fresnel lens (model no. A32681; Edmund Scientific Co., Barrington, N.J.) in all exposures. Glass neutral density filters (Oriol Corp., Stratford, Conn.) were used to adjust the intensity of both types of experimental lights. The duration of the light pulse was automatically controlled by use of a variable timer (Solar Light Co., Philadelphia, Pa.) attached to an iris diaphragm.

In the retinal phototoxicity study, illuminances at the animal's eye level were measured, using a calibrated radi-

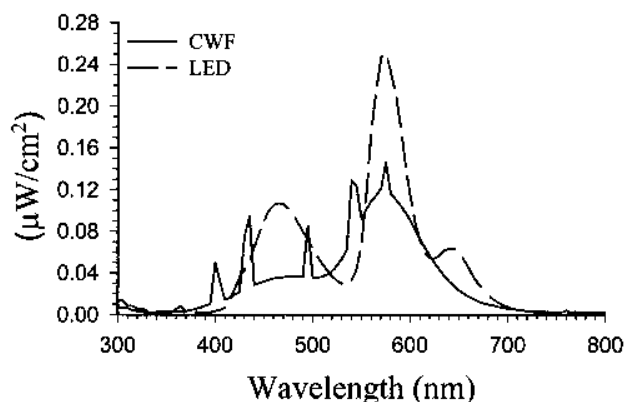


Figure 1. Spectral power distribution curves of light-emitting diode (LED) and cool white fluorescent (CWF) light sources. Energy distribution for LED array is as follows: blue (35.8%), green and yellow (47.2%), and red (17%). Energy distribution for the CWF light source is as follows: blue (34.6%), green and yellow (48.6%), and red (16.8%). Bandwidths with corresponding wavelength ranges and photon energies are as follows: blue (460 to 490 nm/2.53 to 2.70 eV), green (490 to 575 nm/2.16 to 2.53 eV), yellow (575 to 585 nm/2.12 to 2.16 eV), and red (760 to 610 nm/1.63 to 2.03 eV).

ometer (model no. IL-1700; International Light Inc.) with a photometer sensor (model no. SED038, serial no. 2064 using Y filter no. 8990 and W diffuser no. 4945; International Light Inc.). Direct energy was measured in $\mu\text{W}/\text{cm}^2$ by use of the same calibrated radiometer and a radiance sensor (model no. SED038, serial no. 2143 using F filter no. 18629 and W diffuser no. 9089; International Light Inc.). The range of illuminances produced by the photic stimulus during electroretinographic assessment was measured, using the same calibrated radiometer and the photometer sensor.

Melatonin suppression study: All experiments performed during this study were reviewed and approved by Jefferson Medical College Institutional Animal Care and Use Committee and San Jose State University Institutional Animal Care and Use Committee to ensure that experiments minimized any potential pain and discomfort in experimental animals. Adult male Sprague Dawley rats (175 to 200 g, specific-pathogen-free [SPF]; Harlan Sprague Dawley Breeding Laboratories, Indianapolis, Inc.) were maintained on a 12/12-h light/dark (LD) cycle (lights on 0600 to 1800 h) for a minimum of 2 weeks before experimentation. Rats were housed in polycarbonate cages (19 x 10.5 x 8 in.) with microisolator lids, using recycled cellulose fiber animal bedding (Cellu-Dri; Shepherd Specialty Papers, Kalamazoo, Mich.) and were fed Purina Lab Rodent Diet no. 5001 (Purina Mills, Inc., Richmond, Ind.) and water ad libitum. The rats were maintained at an optimal temperature of $21 \pm 1^\circ\text{C}$, relative humidity of 50%, and 10 to 15 room air changes/h. The daily light cycle was produced by use of 40-W CWF light sources (catalog no. F40-CW; Sylvania, Danvers, Mass.), which provided an illuminance of 60 ± 6 lux ($18 \pm 2 \mu\text{W}/\text{cm}^2$) at the animal's eye level. Animal handling was carried out under dim red light produced by a 25-W incandescent bulb behind a Kodak 1A safelight filter (Eastman Kodak, Rochester, N.Y.). The red light had an illuminance of 0.4 to 1.5 lux.

Each of the melatonin suppression experiments (five total, each representing a different exposure intensity) took place between midnight and 2:30 AM on separate days. Each exposure included three groups of eight rats: a group exposed to CWF light, a group exposed to the LED array, and a group maintained in darkness (unexposed control group) by placement in a light-tight box. Therefore, using five illuminances, three treatment groups, and eight rats per treatment group, a total of 120 rats were used in the melatonin suppression study. Each exposure lasted 5 min and occurred while rats were dark adapted. Five light illuminances were tested: 100, 40, 10, 1.0, and 0.1 lux, which correspond to the following LED and CWF irradiances, respectively: 32.0 and 34.8 $\mu\text{W}/\text{cm}^2$, 11.5 and 13.0 $\mu\text{W}/\text{cm}^2$, 3.40 and 3.23 $\mu\text{W}/\text{cm}^2$, 0.30 and 0.36 $\mu\text{W}/\text{cm}^2$, and 0.036 and 0.044 $\mu\text{W}/\text{cm}^2$. Rats were individually exposed to their respective light source (LED or CWF) for 5 min in an 18.5 x 10.5 x 12.5-cm flat black box. The exposure system was a modification of an apparatus described elsewhere (10, 11). After the 5-min light exposure, all rats were transferred to a light-tight holding box where they were kept in darkness for 15 min. The control group was kept for 5 min in the light-tight holding box as well as the additional 15 min. Rats were then euthanized by decapitation, then the pineal gland was removed, placed in a microtiter plate, and frozen at -20°C .

The pineal glands were later assayed for melatonin content, using a modification of the radioimmunoassay (RIA) described by Rollag and Niswender (12). This RIA depends on competition of melatonin in pineal extracts with ^{125}I -labeled melatonin for a limited number of high-affinity binding sites on the anti-melatonin antibodies in the rabbit antiserum. In this procedure, pineal glands were suspended in 200 μL of phosphate-buffered saline (PBS)-0.1% gelatin and were dispersed by use of a micro-ultrasonic cell disrupter (Kontes, Vineland, N.J.). Duplicate 20- μL samples of the sonicate were each placed in an additional 180 μL of PBS-0.1% gelatin. Each duplicate (200- μL volume) was then incubated with 100 μL of [^{125}I] (40,000 cpm/100 μL or 57,000 dpm/100 μL) melatonin analogue and 100 μL of a 1:64,000 dilution of rabbit antiserum containing antibodies against rat melatonin (antibody raised by author MDR on 9/16/74 in rabbit no. R1055) to give a final volume of 400 μL . After a 2-day incubation at 4°C , antibody-bound radioactivity was precipitated by adding 3 ml of cold (4°C) 96% ethanol to the incubation mixture and centrifuging at 1,000 X g for 30 min. The intra- and interassay coefficients of variation were <10% each. Each melatonin assay included an unexposed control group, an LED-exposed group, and a CWF-exposed group from one light illuminance cohort.

For each study (each illuminance), all data from the CWF-exposed, LED-exposed, and control rats were compared, using a one-way Kruskal-Wallis test (this test was used instead of a one-way analysis of variance [ANOVA], because variances in the groups were unequal), where differences between groups were considered significant if $P < 0.05$. Significant differences were further analyzed, using the Student-Newman-Keul's method for multiple comparisons with an experiment-wise alpha of 0.05 for each illuminance.

Retinal phototoxicity study: All experiments per-

formed in this study were reviewed and approved by the San Jose State University Institutional Animal Care and Use Committee to ensure that experiments minimized any potential pain and discomfort in experimental animals. All rats were supplied with Wayne Rodent Blocks-8604 (James Grain, San Jose, Calif.) and water ad libitum. All rats were maintained during the experiments at room temperature ($23 \pm 2^\circ\text{C}$) and at $55 \pm 10\%$ relative humidity, with a total of 15 air changes/h. In the retinal phototoxicity study, the animals were housed under either LED lighting or CWF lighting. Sixteen male Sprague Dawley rats (SPF, 300 to 350 g; Simonsen Laboratories, Gilroy, Calif.) were studied and were housed and tested together in groups of four: two control animals (CWF-exposed rats) and two experimental animals (LED-exposed rats). The CWF-exposed rats were housed in a chamber fitted with two 14-watt CWF bulbs (catalog no. GE F14T12; GE Lighting, Cleveland, Ohio). The LED-exposed rats were housed in a separate chamber fitted with two LED arrays. Individual rats were housed in wire-bottom plastic cages (19 x 10.5 x 8 in.), using Betachips for bedding (Dean's Animal Feeds, Belmont, Calif.) and fitted with aluminum sheet metal shaped into a cylindrical ring (8 in. diameter and 8 in. in height). This design helped provide uniform light exposure to the rat as well as an unobstructed light path. The light sources were mounted from the ceilings of each of the chambers, to ensure that each rat would be exposed directly from overhead to one CWF bulb or LED panel. The desired CWF irradiance was produced by masking the CWF bulbs with aluminum foil as well as adjusting the distance between the CWF bulbs and the rat cages. The desired LED irradiance was established by adjusting the distance between the LED panel and the rat cages. The illuminance used in this study was 100 lux (corresponding irradiances were 28.5 $\mu\text{W}/\text{cm}^2$ from the LED panel and 30.1 $\mu\text{W}/\text{cm}^2$ from the CWF source). The two exposure chambers, one exposing two rats to CWF fluorescent light and the other exposing two rats to LED light, were constructed and maintained so as to keep external light from entering.

Rats were exposed to their respective light source, using 12/12-h LD cycles for 14 days, with lights on at 0700 h. The duration of exposure and the cyclic lighting schedule are important factors when determining the extent of light-induced retinal damage (13). Quantitative changes in retinal function and morphology after toxic light exposure are most reliably measured on the fourteenth day or beyond the start of toxic light exposure (14). All animal handling and maintenance was done under dim red lighting (Kodak Safelight no. 2; Eastman Kodak) that had an illuminance of 0.4 to 1.5 lux.

Electroretinography: On the fifteenth day of the retinal phototoxicity study, rats were moved to a darkroom after dark adaptation overnight (the last dark cycle of the fourteenth day) for scotopic electroretinography (ERG) assessment. Ninety-five percent of the rat retina is composed of rod photoreceptor cells that are best studied in dark adaptation (15). The ERG procedure was performed on rats in the morning, no earlier than 0930 h, to avoid variability associated with photoreceptor disk shedding (16).

Urethane (20% aqueous solution) was administered intraperitoneally (0.15 g/kg of body weight) in rats. Pupils were

dilated by instillation of 10% phenylephrine eye drops. After anesthesia, the rat's right eyelid was held open, and the cornea was protected from drying by application of methylcellulose drops. The ERG assessment performed on each rat was conducted in a Faraday cage without use of animal restraints. A photostimulator producing white light (Grass PS22 Photo Stimulator; Grass Medical Instruments, Quincy, Mass.) was used as a stimulus control to confirm an ERG response in the rats. Monocular recordings from the right cornea were obtained, using a 3-mm-diameter loop of platinum-iridium wire as the recording electrode, a reference electrode on the forehead, and a ground electrode on the cheek. The signals were AC-amplified by use of 0.1-kHz (low frequency) and 1-kHz (high frequency) cutoffs and were displayed on a PC monitor for cursor placement and amplitude measurement, using the computerized BPM-100 ERG recording system (RetinoGraphics, Inc., Norwalk, Conn.). The photic stimulus used in the ERG recordings was a blue LED light placed a standard distance of 9 cm above the right eye to illuminate the entire retina. This method was standardized across rats with elicited ERG waveforms, confirmed with the Grass PS22 Photo Stimulator control. The ERG responses to a series of brief flashes varying in intensity were recorded, starting at the highest intensity and stepping down to the lowest intensity. Each step in intensity represented $1/2$ -log unit. The range of illuminance of the brief flashes was from a maximal 130 lux to 0.1 lux. The range of light intensity was controlled by use of the BPM-100 ERG recording system, which adjusts the power of the stimulus electrically rather than by placement of neutral density filters in the path of the light.

The average of eight responses was obtained at each intensity, with an interstimulus interval of 20 sec. The ERG waveforms were measured, using cursor placement to determine the a- and b-wave amplitudes and implicit times. These values were plotted against the stimulus intensity (intensity-response functions). Statistical evaluation of the data involved use of two-way ANOVA (this test was used because we met the assumption that variances were equal, using the Burr Foster Q-test) with ERG stimulus as a within-subjects factor and light source as a between-subjects factor, where results were considered significant if $P < 0.05$. Stimulus intensity was confirmed by direct energy measurement at the beginning and end of the experiment. On completion of ERG data collection, each rat was euthanized with CO_2 , and the right eye was removed and placed in modified Karnovsky's fixative for histologic examination.

Retinal histologic examination: To determine whether light damage occurred in the retinas of the LED- and CWF-exposed rats of the retinal phototoxicity study, retinal epithelium, rod outer segments, rod nuclei, and total retinal thickness were examined. The quantitative histologic analysis was performed, using seven LED-housed rats from the ERG study, seven CWF-housed rats from the ERG study, and three control rats not subjected to the ERG procedure. The three control rats were not exposed to the experimental chamber environments, but were maintained on a 12/12-h LD cycle of CWF light (250 lux, lights on at 0700 h) in a vivarium for 14 days. Right eyes were fixed by immersion in

modified Karnovsky's fixative (1% paraformaldehyde, 1.5% glutaraldehyde in cacodylate buffer) for at least 72 h. After fixation, each eye was further fixed in 2% osmium tetroxide, bisected on the vertical meridian, and embedded in epon-araldite plastic.

The retinal tissue was sectioned by use of a Sorval JB-4 ultramicrotome (Dupont Instruments, Tucson, Ariz.). Semithin sections were stained with toluidine blue (1% in 1% borax) and were examined by use of a Nikon research microscope with an ocular micrometer attachment. Retinal measurements were made under 100x oil immersion at 270 and 450 μm from the optic nerve head. Retinal pigmented epithelium was examined for the presence of missing areas of retinal pigmented epithelium, vacuolation, and vesicles. The rod outer segment length was measured and observed for any signs of damage and disorganization. Numbers of rod nuclei were counted perpendicular to the retinal pigmented epithelium. Lastly, the thickness of the retina was measured perpendicular to the retinal pigmented epithelium. Statistical evaluation of the data involved use of one-way ANOVA, where differences between groups were considered significant if $P < 0.05$.

Results

Melatonin suppression study: Figure 2 shows the pineal melatonin concentrations for each of the three groups of rats at the respective light exposure intensities. Because each light exposure experiment took place on different days (often separated by weeks), experimental values were compared with those for the respective control groups. Under both experimental light conditions (CWF and LED), at all illuminances examined (100, 40, 10, 1, and 0.1 lux), rats experienced significant melatonin suppression ($P \leq 0.01$ for control versus CWF and control versus LED). Melatonin suppression induced by LED illuminance was not significantly different from that elicited by the same CWF illuminance ($P > 0.05$ for LED versus CWF at all illuminances).

Retinal phototoxicity study: Although all 16 rats completed the 14-day light exposure protocol, two rats (one CWF-exposed and one LED-exposed) had anesthesia complications and could not be used in the ERG and retinal histologic assessments. Therefore, values from 14 rats (7 CWF-exposed and 7 LED-exposed) were used for the ERG data analysis and retinal morphology analysis. Figure 3 shows examples of isolated individual ERG waveforms gathered during our ERG assessment and are presented only to illustrate ERG responses (with their a-waves and b-waves). Figures 4 and 5 are ERG response-intensity curves plotting mean ± 1 SD for the a-wave implicit times and amplitudes for rats of the LED and CWF groups and do not indicate evidence of retinal phototoxicity. Figures 6 and 7 are ERG response-intensity curves plotting mean ± 1 SD for the b-wave implicit times and amplitudes for rats of the LED and CWF groups and do not indicate evidence of retinal phototoxicity. The ERG a-waves were rarely observed at the lower photic stimulus intensities, and were undetectable below the $-1.5 \log_{10}$ intensity in LED- and CWF-exposed animals (Figures 4 and 5). The mean implicit times and amplitudes of the ERG a- and b-waves were not significantly ($P > 0.05$) different for

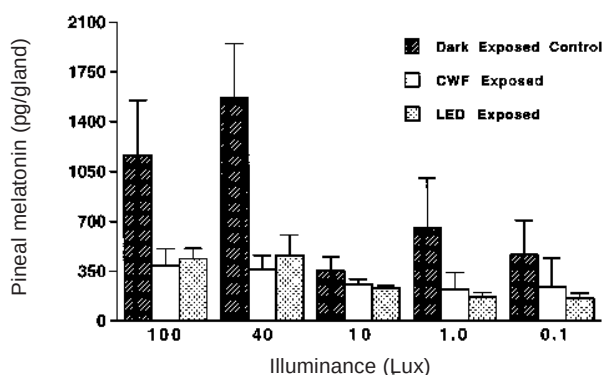


Figure 2. Pineal melatonin concentrations in control (dark adapted), CWF-exposed, and LED-exposed rats at five light intensities. Values are mean $\pm$ SD, $n = 8$ /group.

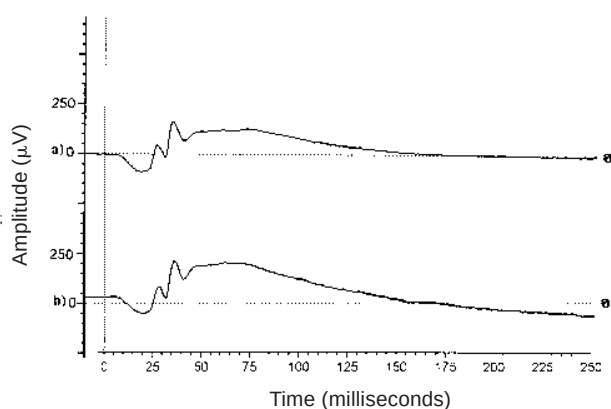


Figure 3. Electretinographic (ERG) waveforms evoked (obtained by use of a monochromatic blue stimulus) from a rat housed under CWF lighting (a) and a rat housed under LED lighting (b).

the two treatment/exposure groups. The ERG stimulus intensity significantly ($P < 0.001$) affected a- and b-wave implicit times and amplitudes, as expected. We expected to see differences between the stimulus intensities because stimulus intensities were $0.5 \log_{10}$ units apart. Lastly, there was no significant ($P > 0.05$) interaction between light source and ERG stimulus for any of the four ERG measurements.

Retinal histologic findings: Evidence of phototoxicity was not found in retinal morphology (Table 1). The retinal pigmented epithelium was intact and did not show vacuolation, whereas rod outer segments were of normal thickness and did not show damage or disorganization. Significant differences were not found among LED, CWF, and control groups in measurements of rod outer segment length, number of outer nuclear layer nuclei, or overall thickness of the retina.

Discussion

Our results indicate that a novel LED light source can suppress pineal gland melatonin concentration in an equivalent manner to a conventional CWF light source at similar intensity levels. Also, we documented that there are no significant differences in retinal function (determined by ERG) or retinal structure (determined by light microscopy) for animals exposed to LEDs, compared with animals exposed to

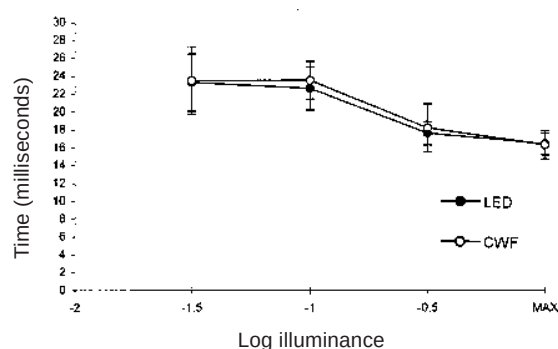


Figure 4. The ERG a-wave implicit times (mean $\pm$ SD, $n = 7$) from rats housed under LED and CWF lighting.

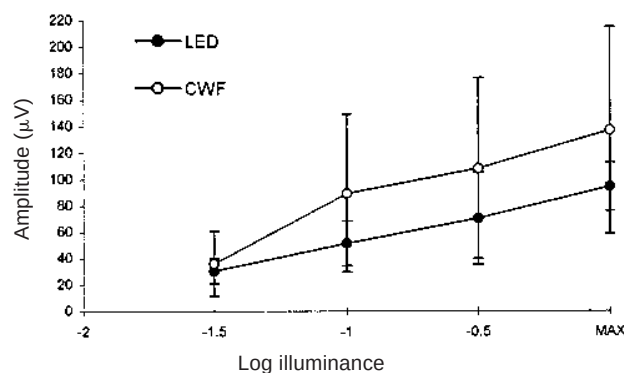


Figure 5. The ERG a-wave amplitudes (mean $\pm$ SD, $n = 7$) from rats housed under LED and CWF lighting.

CWF lighting. In addition, our parallel study, as reported in (5), indicates that LED light maintains normal circadian entrainment, as assessed through behavioral parameters, such as gross locomotor activity, drinking, and feeding. To compare our results with national standards for specifying light in animal housing facilities (9), we have framed the experiments reported here in terms of photometry. We reported our light intensities in radiometric terms ($\mu\text{W}/\text{cm}^2$) for greater usefulness to the research community.

Light suppression of pineal gland melatonin content was used as a bioassay in this study because it is a sensitive measure of the effects of light on the circadian system of rodents and other species. The sensitive nature of the retinal-hypothalamic-pineal axis in the white laboratory rat was initially documented by Minneman and colleagues (17). The exposure of the mammalian retina to light during the night induces neuronal signals in the retinohypothalamic pathway, which in turn increases glucose metabolism in the suprachiasmatic nuclei of the hypothalamus (18). Signals from the suprachiasmatic nuclei are transmitted to the pineal gland, leading to rapid suppression of pineal enzymes and subsequent lowering of pineal gland melatonin content (19). The suprachiasmatic nuclei have been known to be the endogenous pacemakers for circadian rhythms in mammals, including rhythmic pineal gland synthesis and secretion of melatonin (20). This response of the retinal-hypothalamic-pineal axis to light is dependent on the intensity (21) and wavelength (22) of the light. Therefore, the ability of each

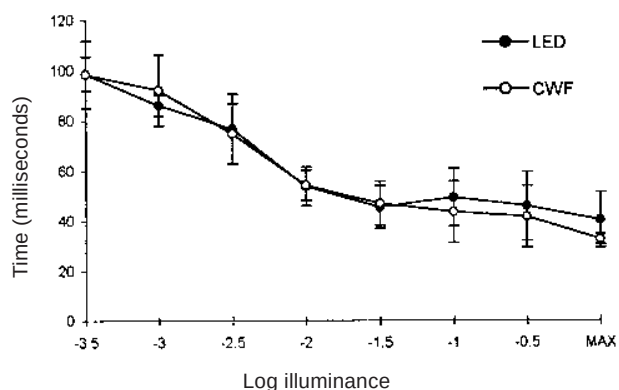


Figure 6. The ERG b-wave implicit times (mean $\pm$ SD, $n = 7$) from rats housed under LED and CWF lighting.

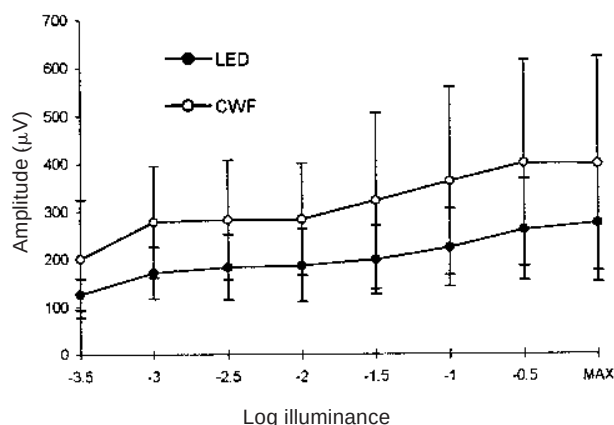


Figure 7. The ERG b-wave amplitudes (mean $\pm$ SD, $n = 7$) from rats housed under LED and CWF lighting.

light source to suppress pineal gland melatonin content at each of the light intensities used provided a meaningful comparison between LED and CWF light. The data from these experiments indicate that there is no significant difference in melatonin suppression between rats exposed to CWF light and rats exposed to LED light at illuminances ranging from 100 lux to 0.1 lux. The significant suppression at the lowest illuminance suggests that even lower intensities of CWF and LED light might suppress pineal gland melatonin content. The ability of LED light to suppress pineal gland melatonin concentration significantly at low illuminances, comparable to that of the CWF light, suggests that it can be equally effective in regulating other aspects of circadian system physiology. Further tests on other circadian parameters are required to confirm this.

Light exposure can cause retinal damage and irreversible loss of photoreceptor cells, resulting in partial or total blindness (2–4, 23, 24). It was especially important to determine whether the LED arrays caused phototoxicity because of the narrow wavelength band in component LEDs, as light damage is produced more readily by some wavelengths of visible light (2). To determine whether the use of LED lighting in animal habitats would have any deleterious effect on the retina, we compared effects of LED animal-habitat lighting and CWF animal-habitat lighting on retinal function, using

ERG assessment. If there was functional damage to the retina, it would be observed by changes in the ERG, which is the evoked mass response of the retina to a flash of light (25). The amplitudes of the individual components of the composite ERG waveform can be related to specific cellular layers of the neural retina and the retinal epithelium (25). The a-wave is the first part of the ERG and is the component produced by the photoreceptor cells (25). The b-wave, which is the most salient fast potential of the ERG of the intact retina, is a result of changes in extracellular potassium ion concentration in Muller cells as a result of on-bipolar excitation (25). Loss of retinal function would be indicated by reduction in amplitude of the ERG a- and b-waves, reflected by reduced sensitivity to light or functional loss. In addition to loss of visual function, retinal phototoxicity may also affect the hypothalamic-pineal axis due to the transmission of signals from the eye to the central pacemaker of physiologic and behavioral rhythms. The results of the ERG assessment indicate that LED light does not reduce photoreceptor sensitivity or disrupt retinal function (Figures 4–7).

Morphologic assessment was performed after ERG functional assessment to determine the presence of any photoreceptor cell damage or death. Past findings have indicated photoreceptor degeneration in albino rats exposed to light intensities >60 lux for 13 weeks, using a 12/12-h LD cycle (26). Another study did not indicate signs of retinal degeneration in rats exposed to 194 lux for 4 to 6 months, using 14/10-h LD cycle (4). The histologic data obtained from our study support the conclusion that 100-lux LED lighting for 14 days, using a 12/12-h LD cycle, does not cause retinal damage (Table 1).

Investigating the effects of light on mammalian physiology involves the discipline of photobiology, the interaction between optical radiation and living organisms. Specifically, photobiology is the study of how the infrared, visible, and ultraviolet portions of the electromagnetic spectrum influence biological processes (27, 28). Light, however, is often understood and described relative to the human visual system, where most individuals discuss it in terms of its apparent color and brightness. This description of light is serviceable for purposes of general communication, but is less useful as a descriptor in photobiology. There are two broad categories of light measurement techniques: radiometric and photometric (29). Radiometry is based exclusively on the physical properties of light, such as its energy and wavelength, whereas photometry is based on the selective responsiveness of the human visual system (29, 30). Radiometric quantification of stimuli is particularly important in photobiological research. A radiometer measures the radiant power of a light source over a defined range of wavelengths, whereas a photometer measures the luminous flux falling on a surface that stimulates the human eye during the daytime. A photometer is simply a radiometer that has filters added to the detector that “shape” the detector sensitivity to resemble the luminance (brightness) response of the human visual response of the “standard observer,” as determined by the Commission Internationale de l’Eclairage (CIE) (30). Thus, photometry is a special branch of radiometry.

Table 1. Retinal morphometrics of light-emitting diode (LED)-exposed rats, cool white fluorescent (CWF)-exposed rats, and control rats

Measure	Mean (and SD) by light source			ANOVA ^a
	Control (n = 3)	CWF (n = 7)	LED (n = 7)	
Rod outer segment length (μm)	24.4 (4.5)	24.2 (6.4)	22.1 (3.2)	0.38, 0.69
Rod nuclei number, outer nuclear layer	9.7 (0.8)	9.3 (1.0)	9.0 (0.9)	0.61, 0.56
Retina thickness (μm)	188.3 (27.9)	210.8 (42.5)	186.1 (32.1)	0.90, 0.43

^aF statistic and P value for (2,14) df

Although the photometric system provides a serviceable nomenclature and measurement technique for describing the light stimuli used in this report, it does not imply that the investigators accept that lux measurements are specifically relevant to circadian and neuroendocrine responses in rats. To the contrary, the photoreceptive physiology in rodents for circadian and neuroendocrine regulation has not been identified (31). It is unlikely that the visual sensitivity of the human eye will be equivalent to the sensitivity of the input physiology of the rodent circadian system. Once again, we have framed these experiments in terms of photometry to compare our results with national standards for specifying light in animal-housing facilities (9). The optimal measurement for circadian and neuroendocrine regulation in animals will be resolved only when the photobiological physiology that mediates these effects of light is clarified.

In conclusion, we found that LED light may be a suitable alternative to CWF light for animal-habitat lighting. Replacing incandescent or fluorescent light sources with LEDs carries a number of advantages, including longer operating life, less mass and volume, less heat production, less power consumption, and higher efficiency (candela/watt of power)(1). In addition to the mechanical and economical advantages, LEDs can be used to produce more precisely timed and spectrally controlled photic stimuli. Light-emitting diode light appears to support normal circadian physiology and does not cause functional damage or morphologic destruction in the retina. Furthermore, we believe the data reported here, using alternate light sources and indicating melatonin suppression at low illuminances (0.1 lux) as well as no retinal phototoxicity under higher illuminances (100 lux), contribute important new information to our understanding of the effects of light on laboratory-animal health and well being.

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