The Influence of Low-powered Family LED Lighting on Eyes in Mice Experimental Model

Mei-Ling Peng[1#], Cheng-Yu Tsai[2#], Chung-Liang Chien[3], John Ching-Jen Hsiao[2], Shuan-Yu Huang [2], Ching-Ju Lee[4], Hsiang-Yin Lin [2], Yang-Cheng Wen[2], Kuang-Wen Tseng[1,2,*]

[1] Department of Ophthalmology, Chung Shan Medical University Hospital, Taichung, Taiwan. ROC
[2] School of Optometry, College of Medical Sciences and Technology, Chung Shan Medical University, Taichung, Taiwan. ROC
[3] Department of Anatomy and Cell Biology, College of Medicine, National Taiwan University, Taipei, Taiwan
[4] Department of Internal Medicine, Taipei Hospital, Department of Health, Taiwan. ROC
[#]Contributed equally.
kuangwen@csmu.edu.tw

Abstract: Ocular tissue damage because of exposure to visible light has been demonstrated by the results of human and animal studies. The short-wavelength visible light between 430 nm to 500 nm (blue light) is especially associated with retina damage. Recently, new powerful sources and relatively inexpensive blue energy of LED (light emitting diodes) family lamps in home illumination are available. The aim of this study is to investigate the effects of illumination source from the low-powered and the conscious spectrum source of LED family lamps on retina tissues. The illumination source of LED family lamps was analyzed from 300 nm to 800 nm using an UV-visible spectrophotometer. In animal experiments, young adult mice were assigned to expose to family LED light for 2h every day ranging 2 to 4 weeks or light environment using LED family lamps for 39 weeks. After LED light treatment, sections of eyes were stained with hematoxylin and examined using histopathology. The data clearly demonstrated irradiation of the white LED is above 400 nm and is not within the ultraviolet light region. However, the analysis of spectrum distribution demonstrated that the family LED lighting exhibited power-peak at 450 nm is within the blue light region. Histological results showed that the photoreceptor layer is significantly reduced in thickness after 4 weeks of LED exposure 2h every day or LED illuminated environment. This study provides important data regarding the efficacy and safety of LED light in family illumination. It is impossible to consider these degenerative changes are related unavoidably part of their mechanism of action or an avoidable toxic effect.

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1. Introduction

Optical radiation includes ultraviolet light (UV) (100-400 nm), visible light (400-750 nm), and infrared radiation (750-10,000 nm). Visible light is referred to as short- (blue), medium- (green), and long-wavelength (red) radiation. Irradiation below 286 nm is absorbed by the stratospheric ozone layer on earth. Thus, we are exposed to wavelengths above 286 nm, most of which fall within the visual light spectrum [1].

Although the primary function of the retina is to receive and is to transduction light from the environment, excessive exposure to light is risky to vision [2]. In addition, excess light has a role in the progression of photoreceptor degeneration, age-related macular degeneration (AMD) and retinitis pigmentosa as determined in a human pubic-health study [3-4]. Photoreceptor death is an unalterable damage and cane cause loss of visual filed and night blindness. UV light does not provide useful vision, instead of harm the retina in acute intense exposures [5-6]. The cornea blocks UV radiation in wavelengths below 300 nm, and the crystalline lens blocks most UV radiation between 300 nm and 400 nm [7]. The absorption properties of the cornea and the crystalline lens contribute to the protection of the retina against the hazards of UV light exposure.

Besides UV light, the visible blue light can cause damage to the eye. Short-wave length blue radiation is believed to cause retinal damage or to contribute to the development of age-related macular degeneration [8-9]. Animal experiments indicate photochemical damage of photoreceptor and retinal pigment epithelial cells after retinal exposure to extreme levels of blue light [10-11]. The high-energy photons create reactive oxygen species, which are deleterious to DNA and to a variety of cellular

organelles, particularly the mitochondria [12-13]. Shorter wavelength light is the most hazardous component of the visible spectrum, and is known to generate reactive oxygen species in the retina [14-15].

Considered the ultimate lamp, the longevity and efficiency of light emitting diodes (LEDs) make them optimal for conserving energy. Recently, advancement in materials and in manufacture has resulted in the commercial availability of LEDs with high luminance. LEDs are used in digital monitors and in illuminating alarm clocks, coffee makers, traffic lights, billboards, and more, may one day be used in lieu of fluorescent lighting in offices and in homes.

With the development of high-efficiency and high-powered LEDs, it has grown possible to use LEDs in lighting. Replacement light bulbs have been made. New powerful sources and relatively inexpensive blue energy of LED lamps in family illumination are available. However, at present, there are no uniform standards at this time for low LED power emissions, and the retinal damage thresholds have not been directly tested. Therefore, this study set up the LED-irradiated animal model to test if any potential risk on the retina of eyes.

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2. Material and Methods

Mice

The common black strain of mice, C57BL/6, was used in this experiment. A total of 40 six-week-old mice were purchased from National Laboratory Animal Center, Taipei, Taiwan. All mice were raised under a 12-hr light/12-hr dark cycle.

LED exposure and study groups

The 40 mice were randomly split into four groups (each contained 10 mice), including (1) white family LED irradiation for a consecutive 2-week period (2 hr/day), (2) white LED irradiation for a consecutive 4-week period (2 hr/day), (3) environmental light source with white family LED lamps (the illuminated light of 12-hr light/ 12-hr dark cycle with white family LED lamps), and (4) blank control (no LED exposure), as summarized in figure 1. All experiments were reviewed and approved by the Animal Care and Use Committee in Chung Shan Medical University.

Spectrophotometry

Spectrum distribution of LED family lamps was analyzed using an UV-visible spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Cambridge, UK) from 300 nm to 800 nm.

Histopathology evaluation

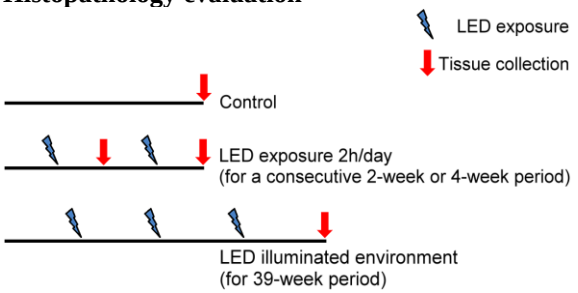

Fig. 1: Schematic summary of the protocol used for analyzing effects of white family LED irradiation on eyes
The response of retinal photoreceptor degeneration after LED exposure was studied by exposed mice to low-powered LED for 2 hr/day (a consecutive 2-weeks and 4-week period) and to illuminated environment of 12-hr light/12-hr dark cycle with white family LED lamps for 39 weeks.

The entire enucleated eyes from light-exposure and control mice were perfused intracardially with a fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS, pH7.4). Tissues were dehydrated through a graded series of ethanol, and then embedded in paraffin. Sections (70 μm-thick) were collected and stained with hematoxylin. Subsequently, sections were mounted and examined under a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

Measurement of the outer nuclear layer thickness and area

The retinal sections obtained after light exposure and unexposed controls were stained with hematoxylin. Using Image Pro Plus image analysis system (Media Cybernetics, Silver Spring, MD, USA), the outer nuclear layer (ONL) thickness and area were measured at 0.5, 1, 1.5, 2, 2.5 and 3.0 mm superior and inferior to the optic nerve disc.

Statistical Analysis

Data are presented as the mean ± SEM. Statistical comparisons were made using a one-way analysis of variance (ANOVA) followed by a Student's t test. The statically significance was assessed at $p < 0.05$.

3. Results

3.1 The spectral irradiance profile of LED source

The emission spectrum of UV/visible lighting ranging from 300 to 800 nm was recorded (Fig. 2). The wavelength within the UV range was not detectable from the lamp of LED source. The spectrum distribution of lighting began to increase at 300 nm and reached sustained maximum transmission at 450 nm. Major peak of the spectrum

distributes was about 0.35 mW/cm2 within the visible wavelength of the blue light. Spectral irradiance profile of LED source showed that the main optical radiation ranging from 430nm to 500 nm.

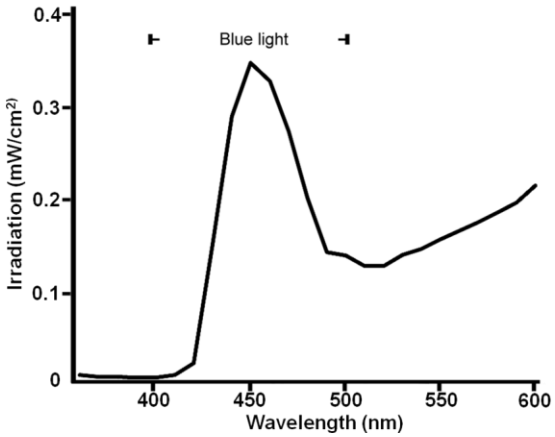

Fig. 2: Spectrum distribution of family LED
Spectrum distribution of low-powered LED is analyzed from 360 nm to 600 nm. Spectra show the peak wavelength of LED lamp is at 450 nm wavelength of blue light region.

3.2 The changes of retinal histology after light exposure from white family LED lamps

We assessed retinal images after LED exposure using morphological methods with hematoxylin staining. In control retinas, well-ordered photoreceptor layer could be seen (Figs 3A and 3B). The ONL thickness was remarkably thinned in LED exposure group compared to control mice (Fig. 3C-3H). For 2 week LED exposure 2 hr every day (Figs 3C and 3D), the light-induced changes in the retinal morphological were less severe than those eyes after changed were significantly severest after 38 weeks exposure (Figs 3G and 3H). In sections from LED exposure mice for 2 weeks and 39 weeks, the ONL thickness was significantly smaller than from control mice (Figs 3E-3H).

3.3 Effects of the LED exposure on light-induced thinning of ONL

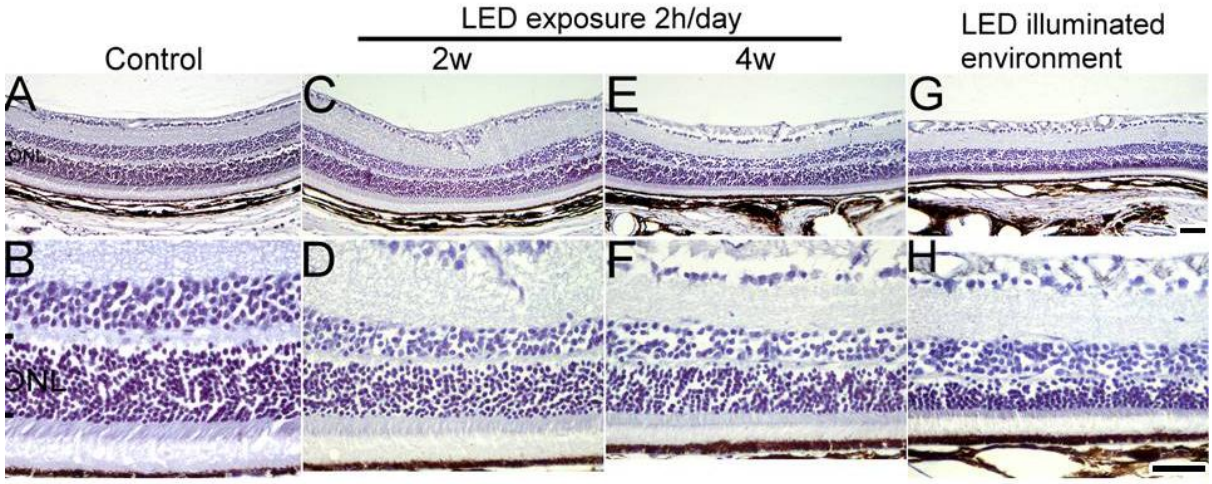

Fig. 3: Representative images of hematoxylin staining for retina section in control and LED-exposure mice
Light micrographs were taken from the mouse retinas. Sections of control and light-exposed retinas stained with hematoxylin. In control retinas (A and B), the outer nuclear layer (ONL) shows the photoreceptor nuclei normally (B). The arrangement of photoreceptor cells in the outer nuclear layer was slightly distorted and the thickness of the outer nuclear layer was decreased after 2 weeks (C and D) and 4 weeks (E and F) exposure. At 39 weeks after light exposure (G and H), with a significant reduction in the thickness of the outer nuclear layer (G), and the photoreceptor cell loss is evident (H). After light exposure, noted that the outer nuclear layer becomes thinner over time. ONL, outer nuclear layer. Scale bars=50 μm.

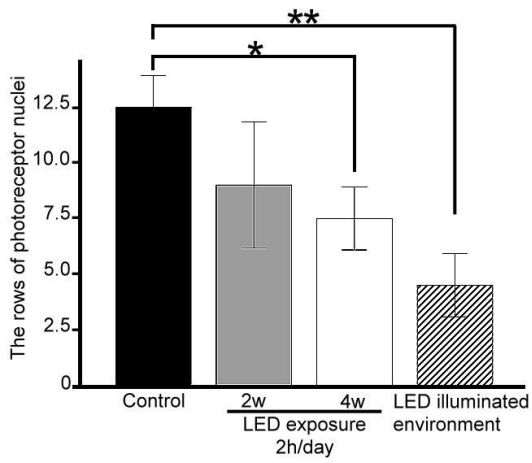

Fig. 4: Quantitative analysis the rows of photoreceptor nuclei in outer nuclear layer after low-powered LED exposure.
In control retinas, the outer nuclear layer shows 12 to 13 rows of photoreceptor nuclei. However, after 2 to 4 weeks period of light exposure, the thickness of outer nuclear layer was become thinner over time. At 39 weeks after light exposure, photoreceptor cell loss is evident, with a reduction in the outer nuclear layer thickness in some region to 4 to 5 rows of photoreceptor nuclei. Compare with control, after 4 weeks exposure and continuous LED illuminated environment, the rows of photoreceptor nuclei were significantly decreased. Data are expressed as means (±S.D.); ** indicate a value statistically different ($p < 0.01$) from the control, and * indicate a value statistically different ($p < 0.05$) from the control.

In control mice, the ONL thickness was identical in the entire retina. In LED-exposed for 2 weeks mice, the rows of ONL was decreased, whereas there was no significant different between in 2 LED-exposed for 2 week mice and in control mice (Fig. 4). After LED exposure for 4 week period, the number of row of photoreceptor cells was scarce in retina (about 7 rows) compared to the control eyes (about 12 rows), and the difference reached statistical significance. The decreased rows of photoreceptor cells with statistical significance were also detectable in the mice after LED illumination for 39 week. These results show that LED exposure decrease the rows of ONL in the retina, and ONL became thinner over time (Fig. 4). Thus, histogram photoreceptor ceel rows clearly showed the effect of retina by the LED exposure.

3.4 Effects of the LED exposure on light-induced cell death of the photoreceptor cells

Sections showed that retinas subjected LED exposure were thinner than control eyes. The retinal thinning was mainly due to the decrease in size of ONL as a consequence of a fall in the number of photoreceptors. In control retinas, the ONL showed more photoreceptors nuclei. After LED exposure 2 hr every day for 2 week and 4 week period, the number of ONL became fewer over time. Moreover, photoreceptor cell loss with a reduction in ONL thickness after LED illuminated environment for 39 week. To determine if the lighting have any damaging effect, the number of photoreceptor nuclei from LED-exposed eyes were compared to those from controls (Fig. 5). By quantitative analysis, the number of photoreceptor cells in ONL from LED exposure 2 hr every day for 2 week was not different from control eyes ($p > 0.05$). However, eyes from LED exposure 2 hr every day for 4 week period and from LED illuminated environment for 39 week, the number of photoreceptor nuclei was significantly fewer than that from control eyes ($p<0.05$).

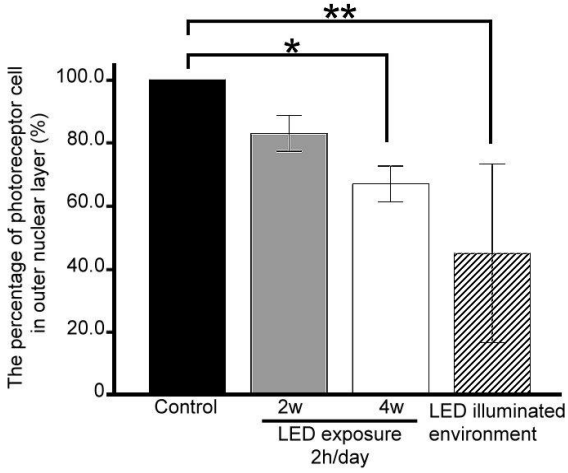

Fig. 5: Quantitative analysis the percentage of the photoreceptor cell number in outer nuclear layer after low-powered LED exposure
In control retinas, the outer nuclear layers were normally observed the photoreceptor cells in outer nuclear layer. However, the number of photoreceptor cells was decreased about 13% and 23% in the outer nuclear layer from 2 weeks exposure and maintain decreased for 4 weeks exposure. A significant decrease about 45% in the number of photoreceptor cells is observed from LED exposure environment for 39 weeks. Data are expressed as means (±S.D.); ** indicate a value statistically different ($p < 0.01$) from the control, and * indicate a value statistically different ($p < 0.05$) from the control.

4. Discussion

It was well known that visible light produce damage to photoreceptor cells of retinal tissues [16]. Histopathology examinations 30 days after lighting indicated that blue LED exposure greater than 60 J/cm^2 caused a significant disruption of photoreceptor cells of retina [17]. It also demonstrated that the continuous spectrum, blue light from LED is a danger to retinas in normal young rhesus monkeys [18]. In our study, the light from low-powered white house LED output mainly falls greater than 400 nm (Fig. 2). Moreover, as showed in the spectrum distribution, it exhibited a power-peak of at 450 nm within the wavelength range of the blue light. Consequently, theses data indicate that white house LED lamps might have additional risk of degenerative factors on photoreceptor cells of retinal tissues after lighting.

As higher-powered blue light from LED is known to induce retinal damage and dysfunction [17], we demonstrated the effect of photoreceptor cells on light-induced photoreceptor degeneration by irradiation from low-powered white family LED in mice with hematoxylin staining. Several studies were indicated that the thickness of the ONL in retina decrease after 1 day [19]. In this study, histopathology study showed significant atrophy of the ONL thickness after 4 week period of LED exposure 2 hr/day, and more serious atrophy over time. Overall, the findings of the present study demonstrate that the thickness of ONL is decrease by low-powered white family LED exposure over time.

Previous studies revealed the action spectrum of retinal phototoxicity increases logarithmically as the wavelength as the wavelength of exposure decrease. The irradiation near 410 nm may contribute to degenerate of the retina [16-18]. However, other results indicated that the ONL area was significant difference after exposure to not only short blue light with peak wavelength 420 nm (ranging 380-500 nm), but also long blue light with peak wavelength 446 nm (ranging 400-540 nm) [20]. In our current study, abnormal thickness of ONL was found after lighting with peak wavelength about450 nm from white family LED. It suggested that the long blue light may induce the retinal injury in eyes, even if the lighting of low-powered output from white house LED. The findings of the present study also demonstrate that the thickness of ONL is decrease by long blue light exposure over time.

Although the primary function of the retina is to receive and transduce light from the environment, excess exposure to light is hazardous to vision [3-4]. Shorter wavelength light is the most hazardous component of the visible spectrum [8-9], and it is known to generate reactive oxygen species (ROS) in the retina [21]. The RPE is especially susceptible to oxidative stress because of its high light, oxygene, fluorophore (e.g. lipofuscin) and membrane lipid (e.g. polyunsaturated fatty acids) levels [22]. Several molecules in the retinal cell are implicated in mediating the phototoxicity of light.

Removing the lens by cataract surgery increases the amount of light exposure that approaches the retina [23]. To compensate for reduced blue light filtering intraocular lens, blue light- and UV-absorbing yellow-tinted intraocular lens were introduced in the 1990s and made with rigid polymethylmethacrylate material. More recently, these intraocular lens were product with foldable silicone or soft acrylic material. However, the significantly protective effects of yellow intraocular lens filter through its blocking of the transmission of light against shorter (420 nm) peak wavelength blue light, but not against longer (446 nm) peak wavelength blue light. The light source from white house LED was detected the450 nm peak wavelength of longer blue light (Fig. 2). Accordingly, the longer blue light from the LED lamps was not filtered through the yellow intraocular lens and might was hazardous to the photoreceptor cells of retina.

5. Conclusion

The present data clearly demonstrated irradiation of the white LED is above 400 nm and is not within the ultraviolet light region. However, the exposure of eye in LED illuminated environment was related to the development of photoreceptor loss. It must be noted that the light illuminations used in the present study as an experimental tool were not fully similar to normal condition that which would impinge upon the retina.

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Corresponding Author:
Kuang-Wen Tseng, Ph.D.
School of Optometry, College of Medical Sciences and Technology, Chung Shan Medical University, No. 110, Jianguo N. Rd., Taichung 402, Taiwan, ROC; Phone: +886-4-24730022, ext. 12137
E-mail: kuangwen@csmu.edu.tw

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