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Article

The Protective Effects of Blue Light-Blocking Films With Different Shielding Rates: A Rat Model Study

Xin Liu[1,2,*], Qi Zhou[1,*], Hui Lin[1,*], Jinzhong Wu[3], Zijing Wu[3], Shen Qu[1], and Yanlong Bi[1]

[1] Department of Ophthalmology, Tongji Hospital Affiliated with Tongji University School of Medicine, Shanghai, China
[2] Department of Ophthalmology, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China
[3] Actif Polarizers Technology R & D Center, Xiamen, Fujian, China

Correspondence: Yanlong Bi, Department of Ophthalmology, Tongji Hospital Affiliated with Tongji University School of Medicine, No. 389, Xincun Road, Putuo District, Shanghai, 200065, China. e-mail: biyanlong@tongji.edu.cn

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Purpose: To examine light emitting diode (LED)-induced retinal photochemical damage and assess the protective performance of blue light-shielding films with different shielding rates in Sprague-Dawley rats (SD rats).

Methods: SD rats were randomly divided into five groups: blank control (group I), white LED illumination (group II), and white LED illumination combined with shielding of blue light of wavelength 440 nm at 40%, 60%, and 80% (groups III, IV, and V). The illumination was 200 lux. All animals underwent electroretinography (ERG), hematoxylin-eosin (H&E) staining, immunohistochemical (IHC) staining, and transmission electron microscopy (TEM) observation after 14 days of dark-adaptation before illumination, after 14 days of cyclic illumination, and after 14 days of darkness for recovery following illumination.

Results: ERG showed retinal functional loss after LED light exposure. However, retinal cell function was partly recovered after a further 2 weeks of dark adaptation. H&E staining and TEM revealed increases in photoreceptor cell death after illumination. IHC staining demonstrated that oxidative stress was associated with retinal injury. Although retinal light injury was discovered in the LED light-exposure groups, shielding 60% of blue light of wavelength 440 nm (bandwidth 20 nm) protected retinas.

Conclusions: Cyclic illumination of low light intensity (200 lux) for 14 days produced retinal degeneration; shielding 60% of blue light may protect retinas from light damage.

Translational Relevance: This study found the effective shielding rate that could protect retinas from light damage when shielding specific narrow-band harmful blue light; thus providing a more normative method for protecting eyes from blue light hazard.

Introduction

Artificial lighting is common in modern society, and the current trend in global lighting indicates a shift toward a new generation of energy-efficient white light emitting diodes (LEDs) with long lifespans.[1,2] However, many current white LEDs emit much more blue light peaking at 440 to 460 nm than conventional lamps[3]; this may have a number of health implications, including retinal photochemical damage and disruption of circadian rhythms.[4,5]

Blue light (wavelength 400–500 nm) has been proven to be the main factor in causing retinal photochemical damage.[3,6,7] However, the harmful waveband of blue light is relatively concentrated; 80% of the damage is concentrated in the 415 to 460 nm frequency band, and the damaging effects peak at 440 nm.[3,8–10] Jaadane et al.[11] observed that blue LEDs emitting light at a wavelength of 449 nm induced higher levels of 8-OHdG and N-Tyr, markers of oxidative stress, in rat retina than blue LEDs emitting light at 467 and 473 nm. Sparrow et al.[12]

observed that 50% shielding of light with a center frequency of 430 nm reduced blue light-mediated retinal pigment epithelial (RPE) cell death by 80%. On the other hand, light in the frequency band 460 to 480 nm proved to be more effective in suppressing melatonin release in many studies, which is involved in the control of cognition, alertness, and circadian rhythm in humans.[4,13,14]

Concerns have been raised regarding the potential harmful risks to human health from LEDs, and many products have been developed to protect against the hazards of blue light, such as blue light-shielding intraocular lens (IOLs), glasses, and films. Previous studies have shown that the use of blue light-shielding films provided retinal protection.[15–18] However, the blue light-shielding films used in these studies adopted broad-spectrum light shielding that may diminish the benefits of blue light. Although some existing products shielding narrow-spectrum blue light, there are few in vivo studies that have used narrow-spectrum light shield to study retinal photochemical damage, and the appropriate blue light-shielding rate still remains unknown. In this study, we used a rat model to investigate the retinal photochemical damage induced by LEDs and evaluate the retinal protective performance of blue light-shielding films with different shielding rates.

Materials and Methods

Animals

Six-week-old male Sprague-Dawley rats (SD rats) were purchased from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). All SD rats received food and water ad libitum. The rats were treated humanely and all procedures were in compliance with Declaration of Helsinki and the Use of Animals in Ophthalmic and Vision Research (ARVO 2013).

Light Source and Blue Light-Shielding Films

White LEDs with a correlated color temperature (CCT) of 6500 K were purchased from Foshan Lighting Co. Ltd. (model, FSL T5; Foshan, China). Three kinds of blue light-shielding films (ACTIF, Xiamen, China) were used, which could shield 40%, 60%, and 80% of blue light with wavelength 440 nm (bandwidth 20 nm) (Fig. 1).

Light Exposure

As shown in Figure 2, all SD rats were randomly distributed into five groups ($N = 12$) using the random number table method: group I consisted of rats kept in darkness and served as a blank control; rats in group II were subjected to unfiltered white LED illumination; and rats in groups III, IV, and V were exposed to white LED illumination with blue light shielding set at 40%, 60%, and 80%, respectively. Blue light-shielding films were placed under the light sources suspended 30 cm above the top of the cages. The illumination at the rats' eyes levels was measured as 200 lux with a light meter (model, LT40; Extech Instruments Corp., Waltham, MA). All rats were kept in a dark environment for 14 days to eliminate effect of lighting in their previous rearing environments. After 14 days of dark-adaptation, light exposure began on day 15 and ended on day 28 and followed a 12-hour dark/12-hour light cyclic routine. Rats were then returned to the dark environment for 14 days of recovery. The SD rats were killed in batches ($N = 4$) by administering lethal intraperitoneal injections of sodium pentobarbital days 14, 28, and 42.

Electroretinography (ERG)

As described in previous work,[19] an ERG (EP-1000 System; Nagoya, Japan) was used to detect retinal electrical responses at three times, 14 days dark-adaptation before illumination, after 14 days illumination, and after 14 days in darkness for recovery after illumination. The rats were dark-adapted overnight and recorded in a darkened room. The SD rats were anesthetized with 2% sodium pentobarbital (20 mg/kg, intraperitoneally) after dark adaptation. Pupils were dilated with 0.5% tropicamide (Wuxi Shanhe Group, Jiangsu, China), and corneas were anesthetized with a drop of proxymetacaine hydrochloride (Alcon Pharmaceuticals Ltd., Puurs, Belgium). Flash ERG responses were recorded with an electrode placed on the cornea. The reference electrode was placed subcutaneously in the anterior scalp between the eyes, and the ground electrode was inserted into the tail. The ERG signals were amplified (×10,000) and filtered (0.1 – 300 Hz) by different amplifiers. The bright flashes were set at $6.325 \times e^{-2}$cd $\times$ s/m^2.

Hematoxylin and Eosin (H&E) Staining

The rats were killed via a lethal intraperitoneal dose of sodium pentobarbital, and the eyes were enucleated. H&E staining was performed after 14

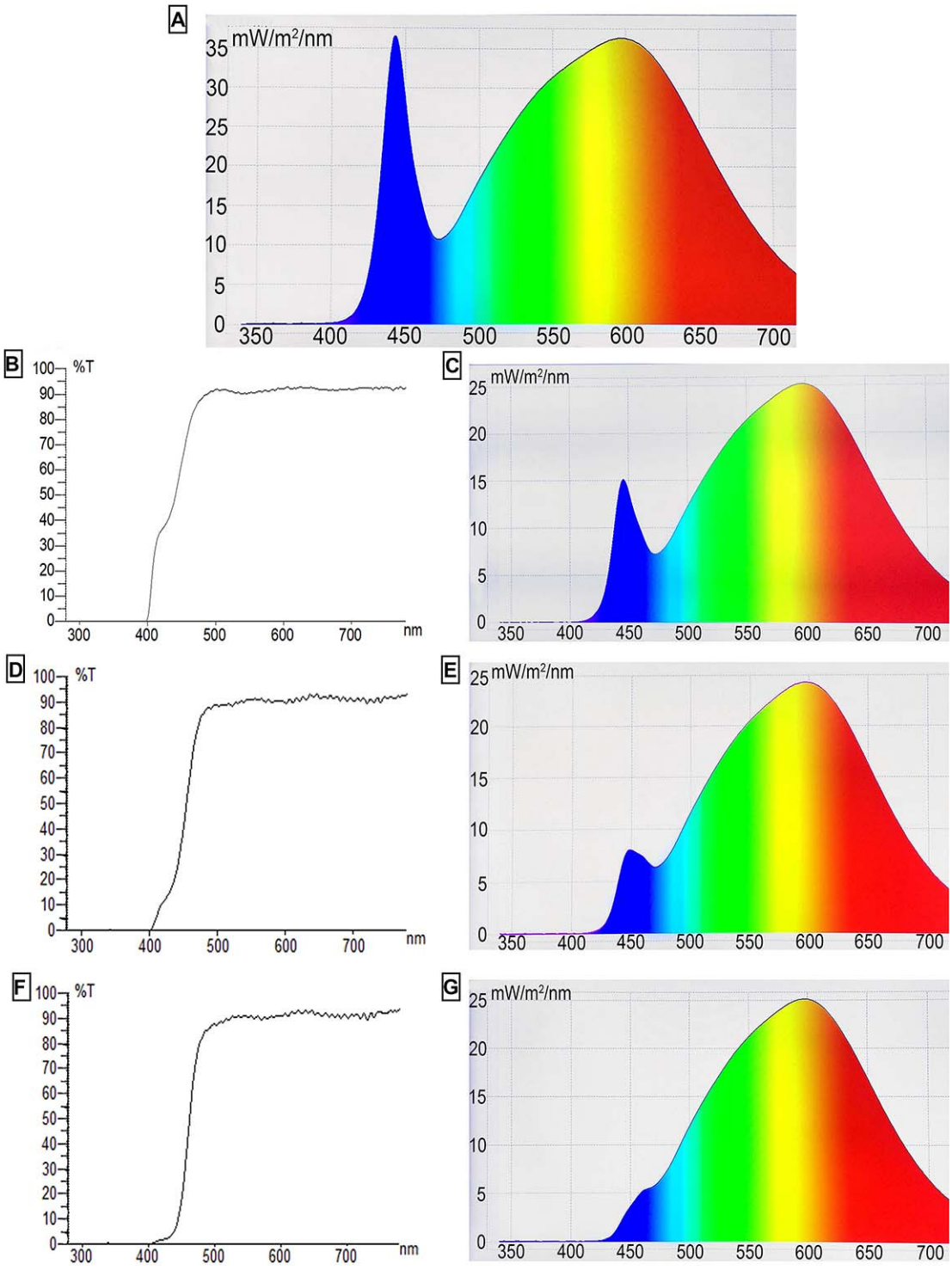

Figure 1. The spectrum power distributions of the light source with and without blue light-shielding films. The white LED peaked at 442 nm for blue (A). The percent transmittance curves show that the films were able to shield blue light of wavelength 440 nm (bandwidth 20 nm) at different rates: 40% (B, C), 60% (D, E), and 80% (F, G).

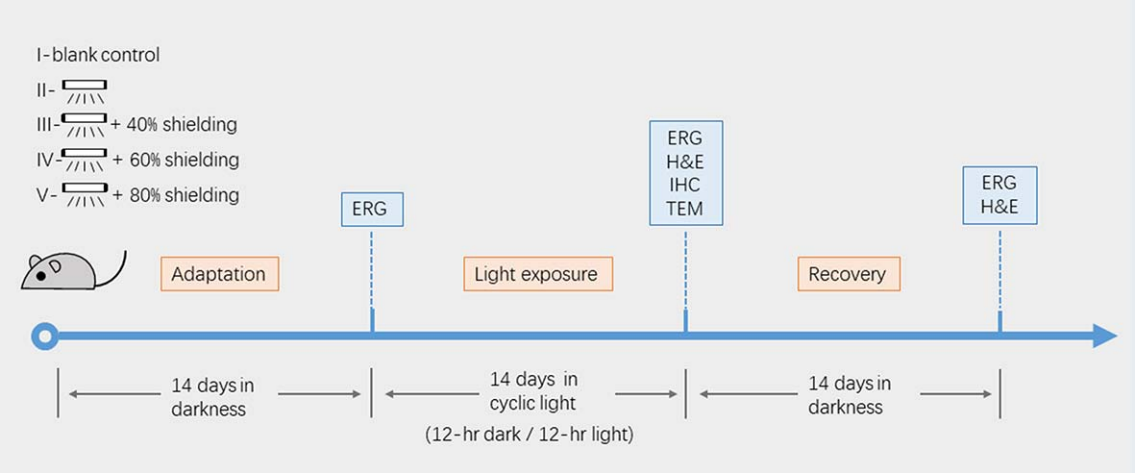

Figure 2. Experimental procedure of the study. The rats were randomly distributed into five groups. Group I is the blank control (remained in darkness); group II to V underwent 14 days dark-adaptation, 14 days cyclic light exposure, and 14 days darkness for recovery. ERG, H&E, IHC, and TEM were detected at different times.

days of light exposure and another 14 days of dark adaptation. As described previously,[20] tissues embedded in paraffin were cut into 5-μm sections and placed on glass slides. After deparaffinization, tissues were stained with H&E and retinal morphology was observed using a light microscope. A histological analysis was performed on the temporal-superior retina. The outer nuclear layer (ONL) and inner nuclear layer (INL) thicknesses were quantified using the ImageJ image processing program.

Immunohistochemical (IHC) Staining

IHC staining was performed after 14 days of light exposure. As described previously,[21] sections of the retina samples were incubated overnight at 4°C with one of two primary antibodies: 8-OHG mouse monoclonal (15A3) antibody (1:200; Seattle, WA) to detect DNA and anti-nitrotyrosine (NT; 1:200; Santa Cruz, CA) to detect proteins. Specific secondary antibodies (anti-rabbit IgG for NT and anti-mouse IgG for 8-OHG) were used, and 4′,6-diamidino-2-phenylindole (DAPI) was used to amplify the signal. The ImageJ image processing program was used to count the relative fluorescence intensity.

Transmission Electron Microscopy (TEM) Analysis

TEM was performed after 14 days of light exposure. As described previously,[22] ultrathin sections (60 nm) were contrasted using uranyl acetate and lead citrate and analyzed with a transmission electron microscope (Philips CM10; Amsterdam, Netherlands).

Statistical Analysis

Data are presented as the mean ± SD. Statistical significance was assessed with a 1-way analysis of variance (ANOVA), and Tukey post hoc tests were used to show the differences between the groups. A P-value < 0.01 was considered to be statistically significant.

Results

ERG Showed LED-Induced Retinal Functional Damage

ERG is a method for evaluating visual function. Some representative ERG response curves for the rats in this study are shown in Figure 3A. The retinas of the LED-exposed rats exhibited a-waves and b-waves of lower amplitudes than the control group, indicating a loss of retinal cell function. However, the peak amplitudes of the a-waves and b-waves recovered after another 14 days of dark adaptation. Increasing the shielding rate of the films appeared to reduce the loss of amplitude and facilitate faster recovery. The amplitudes of the a-waves and b-waves in groups II to V significantly decreased after 14 days of light exposure ($P < 0.01$; Figs. 3B, 3C). After another 14 days of darkness for recovery, the amplitudes of the a-waves and b-waves in groups II to V partially recovered. Additionally, the amplitudes of the a-waves in groups IV and V and the b-waves of groups III to V after recovery were not significantly different from their amplitudes prior to the 14 days of light exposure ($P > 0.01$; Figs. 3B, 3C).

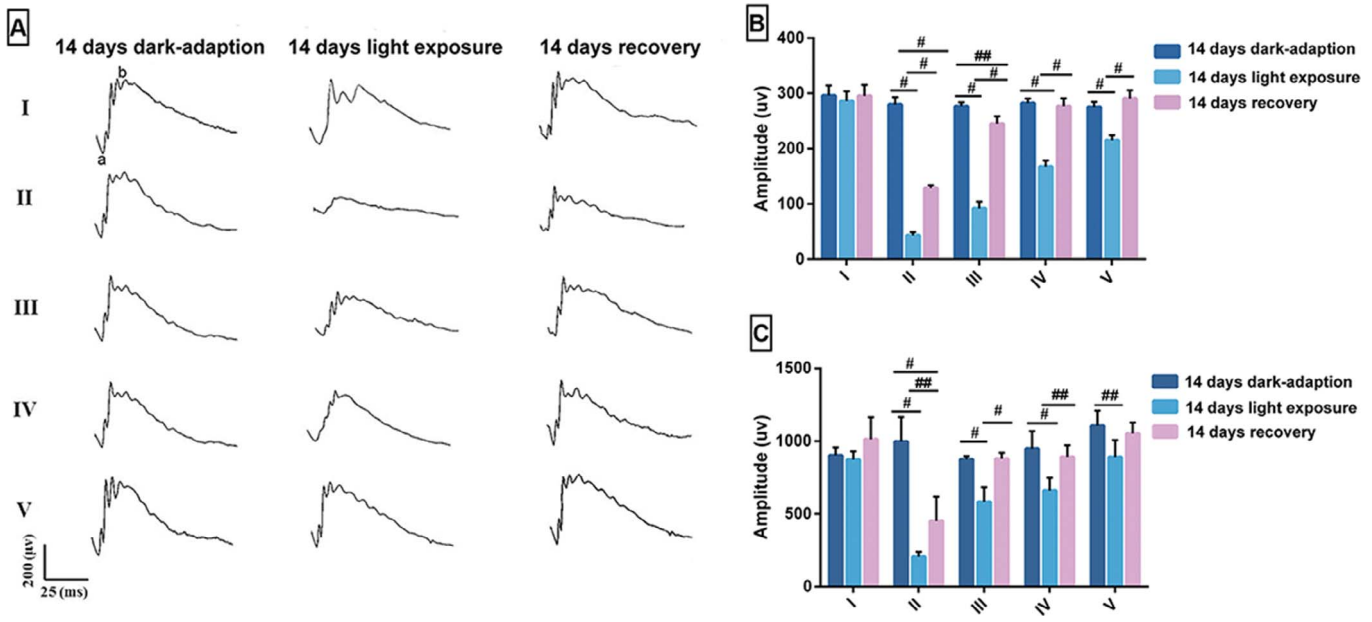

Figure 3. Representative ERG responses (A), ERG a-wave amplitudes (B), and ERG b-wave amplitudes (C) in control rats and rats exposed to LEDs with or without shielding films. Values shown in (B) and (C) are mean ± SD ($N = 4$ for each group at each measurement stage). ##$P < 0.01$ and #$P < 0.001$, compared between each measurement stage.

H&E and TEM Showed LED-Induced Retinal Morphological Changes

As shown in Figure 4A, the control group had clear layers and retinal structure. After 14 days of light exposure, the ONL in group II became thinner and disorganized, and more hyperchromatic nuclei cells were observed. The structures of the retinas in group III were slightly disordered, ONL thickness was thinner, and the cell was swollen. The structures of the retinas in groups IV and V were organized, and ONL thicknesses slightly decreased. The ONL thickness in groups II to V decreased to varying degrees in comparison with group I, but the decrease of ONL thicknesses in groups IV and V were lower than in group II or group III ($P < 0.01$; Figs. 4A, 4B). No significant difference was found in the INL thickness between all groups ($P > 0.01$; Fig. 4C). After another 14 days of darkness for recovery, the retinal structures in groups II to V were similar to that observed at the end of light exposure (Fig. 4D). Additionally, the ONL and INL thicknesses were not found to be different from the end of light exposure ($P > 0.01$; Figs. 4E, 4F).

As shown in Figure 5, nucleolar damage, including karyolysis and pyknosis, was especially pronounced in groups II and III, when compared with the intact structures and regular arrangements of the photoreceptors in group I. Disruptions of the inner segments (ISs) and the outer segments (OSs) were observed and especially pronounced in group II. This nucleolar damage was observed to decrease with the increasing levels of shielding provided for groups III to V.

IHC Staining Showed LED-Induced Retinal Biochemical Changes

Blue light-induced retinal damage is possibly related to oxidative stress, in order to explore this, we used 8-hydroxyguanine (8-OHdG) to investigate DNA oxidation and NT for protein oxidation. The IHC staining for 8-OHdG revealed only a small amount of 8-OHdG expression in group I, mainly located in the ganglion cell layer (GCL) and INL (Fig. 6A). After 14 days of light exposure, the expression of 8-OHdG in groups II, III, and IV increased, mainly in the GCL, INL, and ONL ($P < 0.01$; Figs. 6A, 6B). However, the expression of 8-OHdG in groups III, IV, and V declined gradually ($P < 0.01$; Figs. 6A, 6B). No significant difference was found in the expression of 8-OHdG between group I and group V ($P > 0.01$; Fig. 6B).

The IHC staining for NT showed minimal expression of NT in the INL in group I (Figs. 6D, 6E). After 14 days of light exposure, the expression of NT in groups II, III, and IV increased, mainly in the INL ($P < 0.01$; Figs. 6C, 6D). However, the expression of NT in groups III, IV, and V declined

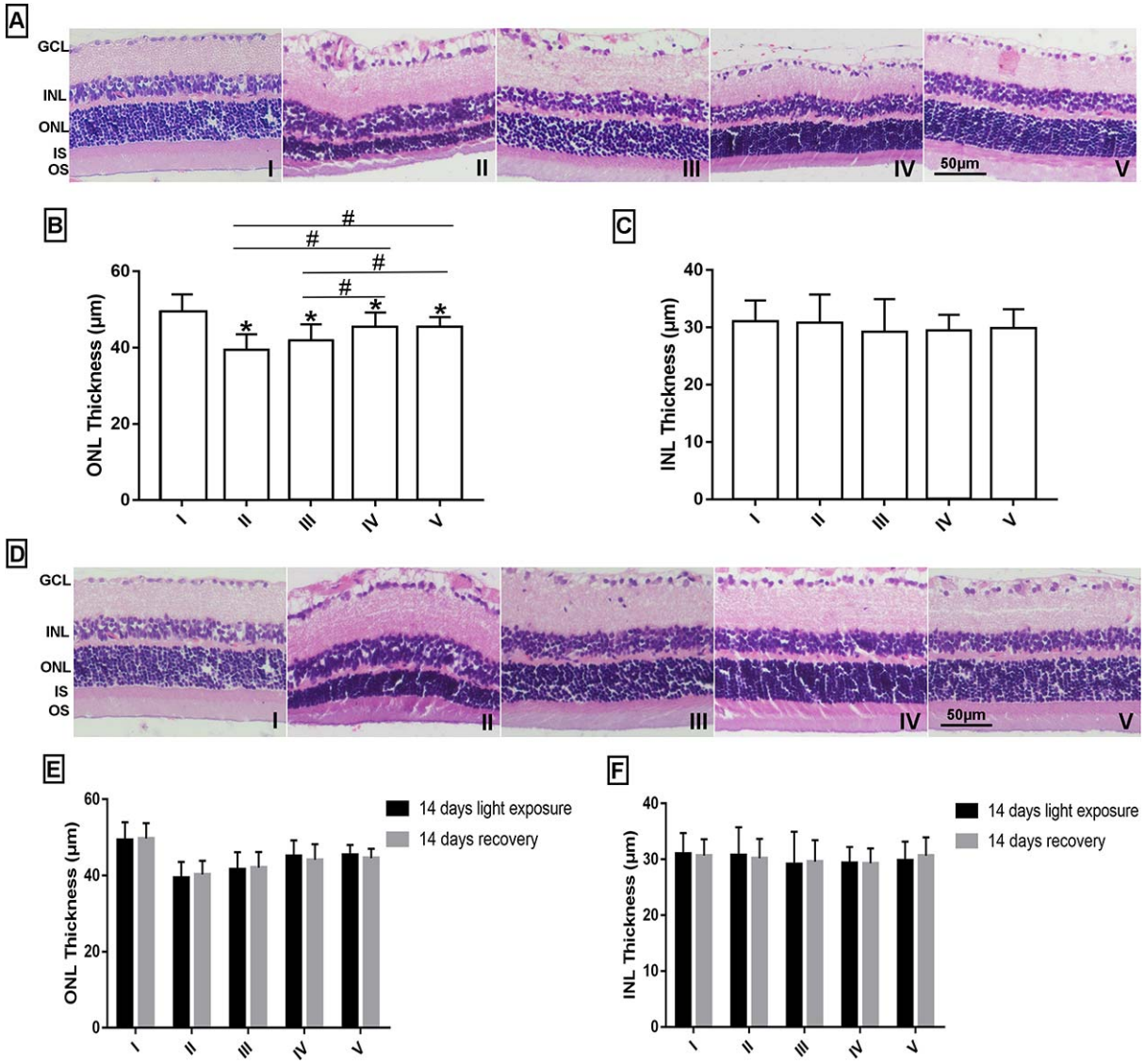

Figure 4. H&E staining of representative retinal tissue sections after 14 days of illumination (A), in which ONL (B) and INL (C) thickness were measured. H&E staining of representative retinal tissue sections after 14 days of recovery (D). The change of ONL (E) and INL (F) thicknesses were measured after 14 days of recovery. Values shown in (B), (C), (E), and (F) are mean ± SD ($N = 4$ for each group at each measurement stage). Comparisons were made between groups I and V in (B) and (C). Comparisons were made between the end of light exposure and 14 days recovery in (E) and (F). #$P < 0.001$, compared between groups II to V. *$P < 0.001$, compared with group I.

gradually ($P < 0.01$; Figs. 6C, 6D). No significant difference was found in the expression of NT between group I and group V ($P > 0.01$; Fig. 6D).

Discussion

LEDs are expected to provide the majority of domestic light in the near future. Certain levels of LED light exposure may induce retinal damage. Light-induced photochemical damage causes photoreceptor cell death, the severity of which depends on the light intensity, exposure time, and wavelength.[23] In order to reduce a series of physiological and behavioral problems that have been introduced by broad-spectrum blue light-shielding methods, we have distinguished between harmful (415–460 nm) and beneficial blue light frequency bands (460–480 nm); we concentrated shielding within the harmful band and retained the beneficial band. To accomplish this, we used blue light-shielding films with different shielding rates of wavelength 440 nm (20-nm bandwidth).

In this study, retinal damage occurred after 14

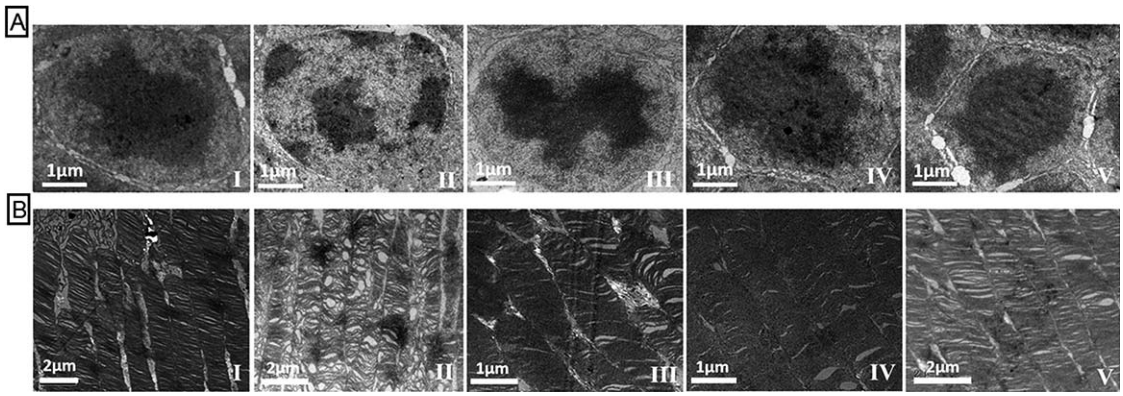

Figure 5. TEM photomicrographs showing the changes of the nucleus of the ONL (A), IS, and OS (B) of retinal photoreceptors in control rats and rats exposed to LED with or without shielding films after 14 days light exposure. The nuclei of photoreceptors were uniformly stained and the nuclear membrane was intact (A–I). The structures of the inner and outer discs were clear and arranged regularly. The IS and OS were closely connected (B–I). After 14 days of light exposure, the photoreceptors were damaged to varying degrees, and damage included karyolysis and pyknosis (A–II). Vacuolated outer discs, swelling, and dissolved mitochondria were also obvious (B–II). The morphologies of most of the nuclei of the photoreceptors were normal, and only part of the nuclei was deformed (A–III, IV, V). The outer discs were intact, the gap between the discs increased slightly, and the swelling was minimal (B–III, IV, V).

days of cyclic exposure to domestic LED light, resulting in changes of ERG, cell structure, and ONL thickness; these results may be related to oxidative stress within the retinal tissue inconsistent with previous findings.[24–31] Moreover, we have shown that blue light shielding can reduce photochemical damage, and greater protection can be obtained with higher shielding rates.

ERG comprehensively measures retinal potentials reactions induced by short flashes, and can therefore reflect biological changes in eyes. It is currently a widely accepted evaluation of visual function for clinical diagnosis and basic research.[5,32] A-waves were derived from retinal photoreceptors and pigment epithelial cells, and b-waves were originated form retinal bipolar cells and Müller cells in the inner retina. The significant decrease in the amplitudes of the a/b–waves in the ERG results indicated loss of retinal function after LED light exposure. In this study, we found that the reduction in the amplitudes of a-waves in the experimental groups was more severe than that of the b-waves, demonstrating that light causes greater damage on the outer layer of the retina (Fig. 3). This finding is in accordance with the morphological results that showed that ONL thickness decreased significantly while INL thickness did not (Fig. 4). Increasing the shielding rate of the films reduced a- and b-wave amplitude loss and facilitated speedier recovery. Additionally, the amplitudes of the a-waves in groups IV and V and the amplitudes of the b-waves in groups III to V recovered to pre-exposure levels. This indicated that shielding more than 60% of blue light could be helpful in ensuring recovery of visual function.

As shown in Figure 4, after 14 days of light exposure, the ONL thickness reduced and the retinal became disordered especially in groups without films or with films of lower shielding rates. However, increasing the shielding rates of the films resulted in smaller changes in the ONL thickness and retinal structure; this finding was also verified by the results from the TEM photomicrographs (Fig. 5). The reduction of ONL thickness was significantly lower, and the cell structure was similar with the blank control when more than 60% of the blue light was shielded, indicating that this level of shielding provided effective protection. There was no significant difference between shielding blue light at 60% or 80%, suggesting that a similar protective effect was achieved. Although ERG readings recovered to varying degrees after 14 days of darkness for recovery, the ONL thickness was not significantly different when compared with the end of light exposure. This suggests that the recovery of a- and b-wave amplitudes in ERG may be relative to the functional compensation of the residual cells.

Previous studies have suggested that blue light-induced retinal photochemical damage could be related to oxidative stress within the retinal tissues.[5,9,33] Mitochondria are the main sources of oxygen free radicals under blue light illumination. Under aerobic conditions, the blue light stimulation of the retina initiates an oxidation mechanism; that generates reactive oxidative species (ROSs). Further-

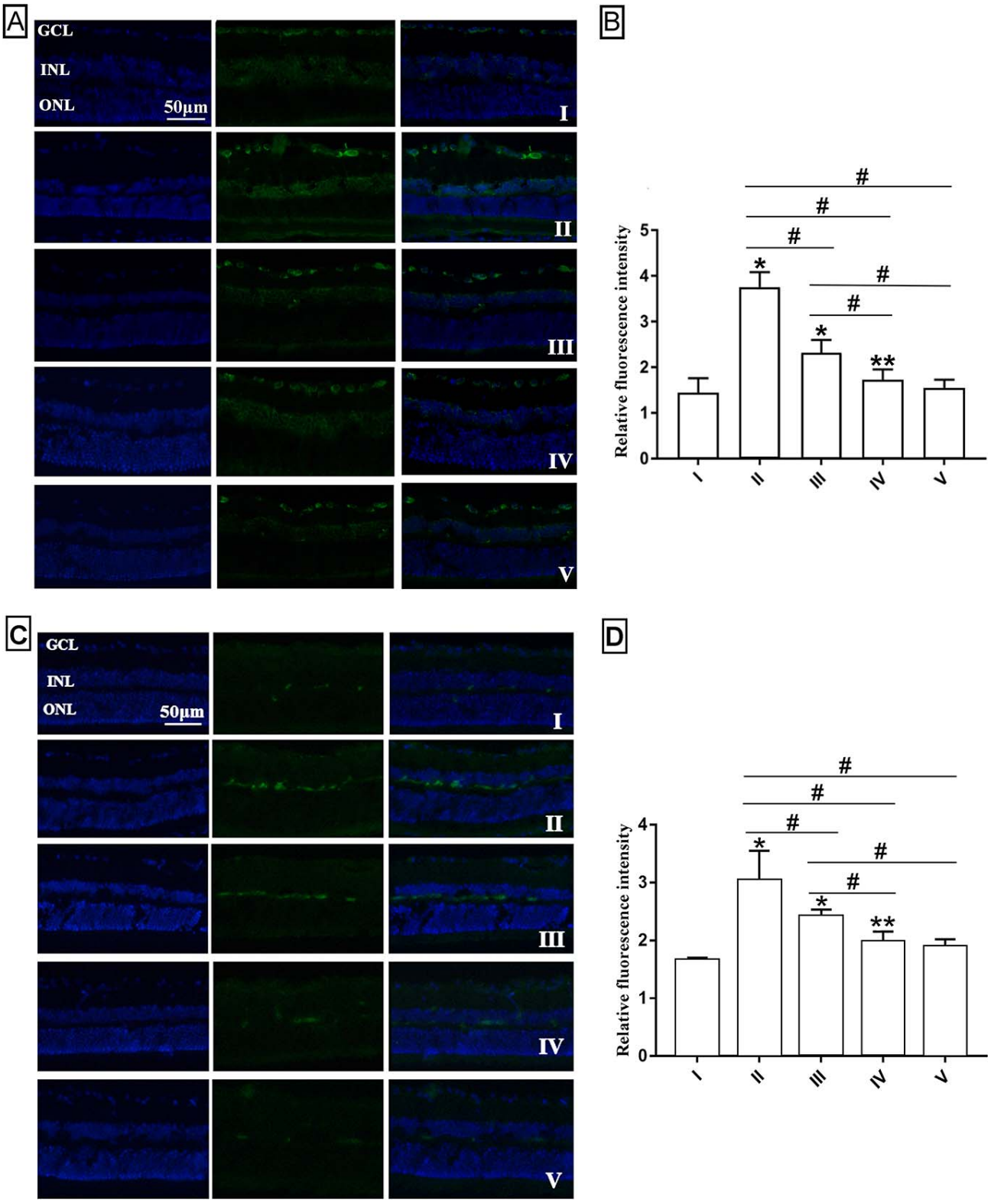

Figure 6. Photochemical damage shown by immunofluorescent staining using 8-OHdG to detect DNA adducts (A) and NT to detect protein oxidative damage (C) in retina in control rats and rats exposed to LEDs with or without shielding films after 14 days of light exposure. The relative fluorescence intensity of 8-OHdG (B) and NT (D) is reported as mean ± SD ($N = 4$ for each group at each measurement stage). Comparisons were made between group I to V in (B) and (D). #$P < 0.001$, compared between groups II and V. **$P < 0.01$ and *$P < 0.001$, compared with group I.

more, ROSs damage mitochondrial DNA (mtRNA) and proteins, and causes apoptosis of photoreceptor cells and pigment epithelial cells.[34] ROSs are difficult to detect because of their chemical properties. However, 8-OHdG, a widely used biomarker of oxidative DNA damage, may be used as a marker of oxidative stress.[35,36] This has been used in conjunction with NT, a stable biomarker for protein oxidative damage,[37] to detect oxidative stress within the retinal tissues in this study.

We found that the LED-exposed rats exhibited higher levels of 8-OHdG immunostaining mostly in the INL and GCL of the retina (Fig. 6A), and this is consistent with research by Jaadane et al.[11] Additionally, Min et al.[38] found that after illumination with light of an intensity of 600 lux for 12 hours blue light inhibited the proliferation of human RPE cells better than red and white light, and the expression of 8-OHdG increased predominantly in the cytoplasm. This study has also shown that the expression of 8-OHdG mainly occurs in the cytoplasm (Fig. 6A), suggesting that mtRNA damage induced by light-induced retinal oxidative damage is more severe than that of nuclear DNA (nDNA) damage. The increased susceptibility of the mtDNA to light damage compared with nDNA was also shown by Godley et al.[39] This may be due to the location and structure of mtDNA; mtDNA is closely located to the inner mitochondrial membrane where ROSs are generated, and unlike nDNA, mtDNA is not protected by histone proteins.[40] We also observed that the expression of NT increased after light-exposure as found by Shang et al.[5] Our results have shown that as the blue light shielding rate increases, the expression of NT gradually decreases (Figs. 6C, 6D). The expression of 8-OHdG and NT was not significantly different from the blank control when 80% of blue light was shielded (Figs. 6B, 6D). In addition, no significant difference was found in photochemical damage between group IV and group V after light exposure (Figs. 6B, 6D), suggesting that shielding 60% and 80% of blue light had a similar protective effect on the retina.

The shielding of narrow-band harmful blue light is likely to be the emphasis of the emerging field of blue light shielding in order to maximize the benefits of blue light. This study investigated the effectiveness of films that were able to shield blue light of wavelength 440 nm (bandwidth 20 nm) at rates of 40%, 60%, and 80%. The protective effect of these films was obvious, especially when more than 60% of the blue light was shielded; 60% may be sufficient since there was no significant difference between shielding at 60% and 80%. Further studies should be completed using human retina to investigate the appropriate shielding rates for humans because of the acknowledged differences in biology and physical environments of rats and humans.

Conclusions

In conclusion, cyclic illumination of low light intensity (200 lux) for 14 days produced retinal degeneration in SD rats. Our results showed that a shielding rate higher than 60% for blue light of wavelength 440 nm (bandwidth 20 nm) can effectively protect retinas from light damage.

Acknowledgments

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*XL, QZ, and HL contributed equally to this work.

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