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Original Article

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Changes in Retinal Structure and Function in Mice Exposed to Flickering Blue Light: Electroretinographic and Optical Coherence Tomographic Analyses

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The harmful effects of blue light on the retina and health issues attributed to flickering light have been researched extensively. However, reports on the effects of flickering blue light at a frequency in the visible range on the retina are limited. This study aimed to non-invasively investigate the structural and functional changes in mice retinas following exposure to flickering blue light. BALB/c mice were subjected to non-flickering and flickering blue light, and changes in the retinal function and structure were assessed using electroretinography (ERG) and spectral-domain optical coherence tomography (SD-OCT), respectively. Retinal damage progression was monitored on days 3, 7, 14, and 42 following light exposure. Significant reductions in scotopic and photopic ERG responses were observed on day 3 ($p<0.05$). On day 7, the non-flickering and flickering groups demonstrated different functional changes: the flickering group showed further ERG response reduction, while the non-flickering group showed no reduction or slight improvement that was statistically insignificant ($p>0.05$). A similar trend lasted by day 14. On day 42, however, the difference between the non-flickering and flickering groups was significant, which was corroborated by the normalized amplitudes at 0, 0.5, and 1 log cd s/m^2 ($p<0.05$). Quantitative and qualitative SD-OCT assays revealed more severe and progressive retinal damage in the flickering group throughout the study. Flickering blue light causes more persistent and severe retinal damage than non-flickering blue light and may be a risk factor for retinal degeneration even at frequencies as low as 20 Hz.

Key words: Blue light, Flicker fusion, Retinal degeneration, Electroretinography, Optical coherence tomography

INTRODUCTION

Humans are increasingly exposed to light-emitting diodes (LEDs) through various electronic digital devices such as smartphones. Potential harmful effects of LED lamps on the eye have been reported, particularly owing to their high blue wavelength emission [1, 2]. Several studies have reported that prolonged exposure to blue light (400~500 nm) can cause oxidative stress in the retina, leading to significant damage to retinal tissues, especially retinal pigment epithelium (RPE) cells [3, 4], and photoreceptor death [5]. Blue light exposure is considered a risk factor for age-related macular degeneration (AMD) [6, 7], a common form of retinal degeneration (RD) that leads to permanent vision loss and eventual blindness in older individuals [8, 9]. The blue LED-induced photoreceptor degeneration model has been widely employed to mimic AMD in mice because it can lead to photoreceptor death, as demonstrated in previous studies [10, 11]. Blue LED-induced RD in albino mice has been employed in several previous studies that explored the mechanism of oxidative stress and repeatedly verified the dangers of blue light to the retina [12,

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13]. Pigmented mice have also been used as models of RD induced by blue light [14]. While blue light is effective in inducing RD, sensitivity to light varies in albino and pigmented mice, which is attributed to differences in the maturation of their visual systems that are related to melanin. In C57BL/6J mice, the melanin-rich pigment epithelium may slow the rate of retinal damage caused by excessive light exposure [15].

A flicker is defined as a rapid change in the light output of a lamp. In addition to the rapid change, the frequency of this change is important. Steady light is referred to as zero flicker. The critical flicker fusion frequency (CFF) is the frequency at which the flickering light ceases to be visible and begins to be perceived as a steady light. CFF has been used in several fields of study on dementia [16-18], cognitive functioning [19-22], and visual perception disorder [23]. Several studies on CFF have been limited to perceptive and cognitive performance associated with neurophysiology or neuropsychology, and knowledge related to retinal disease is limited [24-26]. Recent reports have revealed an association of unstable flickering LED lights with several diseases, regardless of the wavelength of the light. Flickering light combined with blue light emitted by several digital devices can yield an even more threatening environment for the retina, resulting in various potential health concerns in humans, including fatigue, eye strain, blurred vision, and headaches [24, 27, 28]. However, few studies have reported on the effects of visible flicker at frequencies around the CFF on retinal diseases, including AMD and RD that can occur with continuous exposure to blue light at such frequencies [29, 30]. Thus, it is necessary to ascertain whether blue LED light emitted at frequencies as low as 20 Hz affects the retinas of mice.

In this study, we induced retinal damage in albino and pigmented mice by exposing them to excessively flickering blue light and non-invasively assessed the changes in retinal structure and function. The use of electroretinography (ERG) and spectral-domain optical coherence tomography (SD-OCT) allows the simultaneous observation of changes in retinal structure and function. This study aimed to determine whether flickering light causes more severe damage than non-flickering blue light through non-invasive quantitative and qualitative evaluation of functional and structural changes in the retina.

MATERIALS AND METHODS

Animals

Six-week-old male BALB/c (n=8) and C57BL/6J mice (n=20) were used for this study. All animals were housed under temperature-controlled conditions with a 12-hour light/dark cycle. The research protocol was approved by the Institutional Animal Care and Use Committee of the School of Medicine, Catholic University of Korea (approval number CUMS-2022-0152-04).

Exposure to blue light

The procedures used to expose the mice to blue LED light are described in our previous studies [10, 11, 31]. The mice were placed in a dark room for at least 12 h before blue LED exposure, and their pupils were dilated using Mydrin P (Santen Pharmaceutical Co., Osaka, Japan) under low-intensity red light 15 min before LED exposure. The non-anesthetized BALB/c mice were exposed to 1500-lux non-flickering or 20-Hz flickering blue LED (460±10 nm) light for 2 h in cages with reflective interior walls. The C57BL/6J mice were damaged similarly, except that they were exposed to a 1950-lux blue LED for 3 h on three consecutive days. The illuminance intensity of the flickering blue light was measured using the same LED light meter (TM-201L; Tenmars Electronics Co., Taipei, Taiwan) used for the stable blue light, which was set as

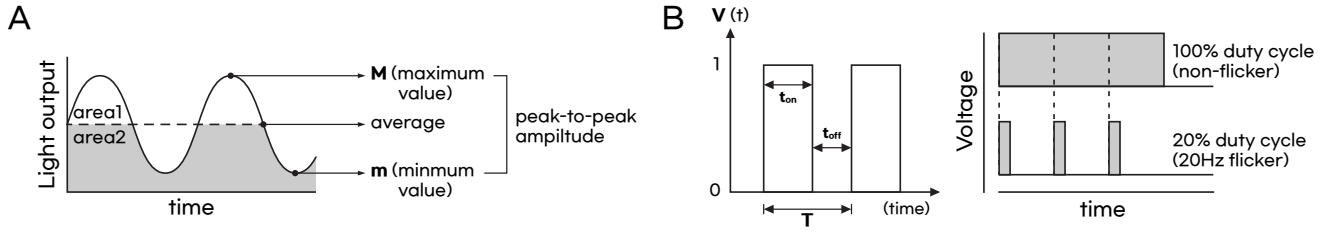

Fig. 1. Flicker quantification and duty cycle of 20 Hz flicker used in the study. (A) Two methods for quantifying flicker are presented with a drawing of a typical sine wave-like pulse. The percent flicker is the simplest to calculate showing the percentage difference between the minimum and maximum light output ($percent\ flicker = 100\% \times \frac{M-m}{M+m}$), while the flicker index is more indicative of changes over time and harder to manipulate ($Flicker\ index = \frac{area1}{area1+area2}$). (B) The blue light stimulator used in our study generates a flicker pulse according to duty cycle, which is the amount of time the signal is in the high (on) state as a percentage of the total time it takes to complete a cycle ($Duty\ Cycle = \frac{t_{on}}{T} \times 100\%$). The duty cycle is expressed as a percentage, where a 100% duty cycle is the same as the signal being fully turned on with maximum voltage, and a 0% duty cycle is the same as the signal being grounded. The 20-Hz used in our experiment is equal to 0.2 when the total pulse duration is assumed to be 1, thus it can be calculated to provide 20% of the total amount of luminous flux generated by non-flickering.

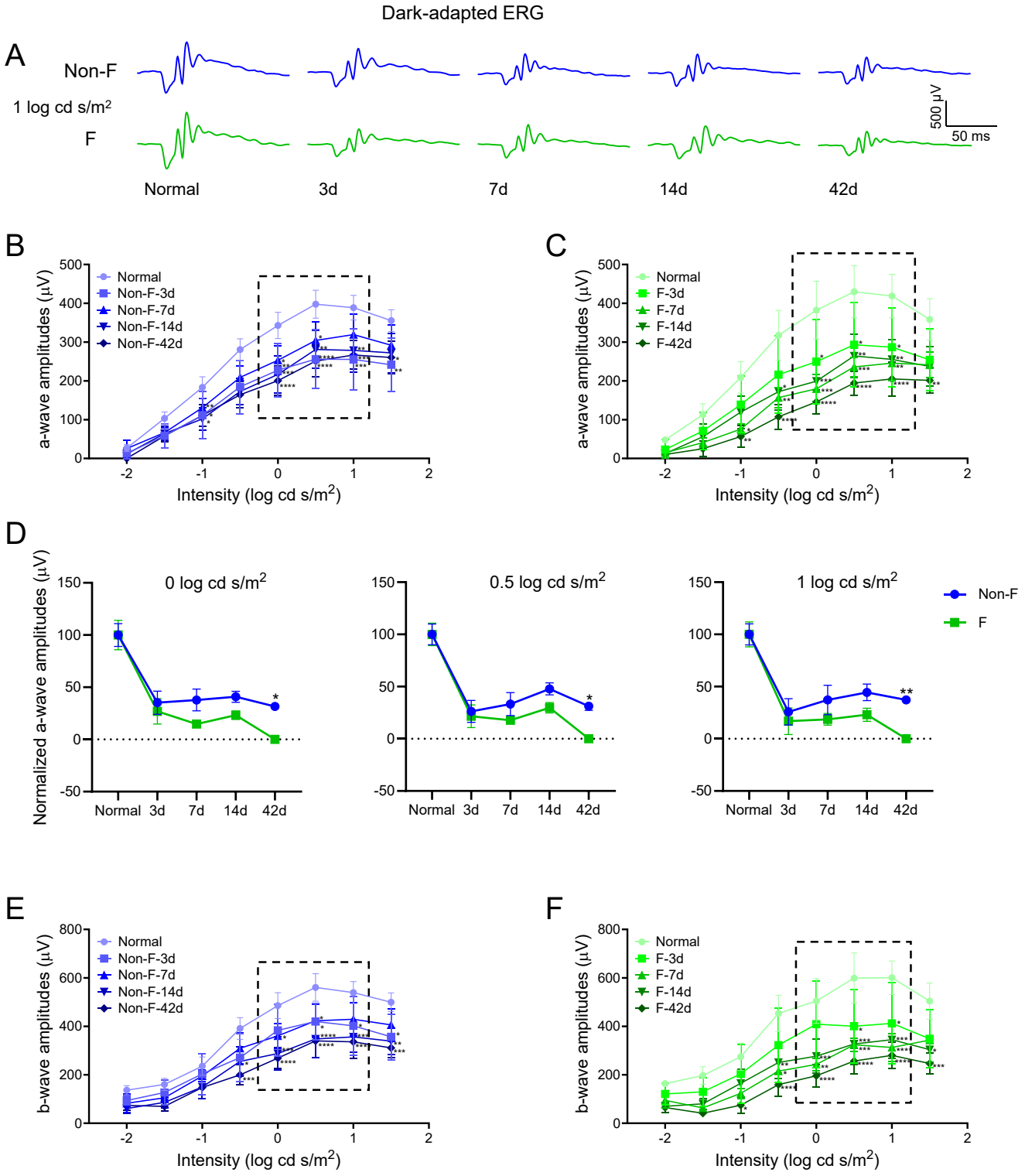

Fig. 2. Dark-adapted amplitudes of a- and b-waves of BALB/c mice after blue light-emitting diode (LED) light exposure. (A) Representative electroretinography (ERG) waveforms before injury and blue LED exposure at 3, 7, 14, and 42 days. The intensity of the stimulus flashes was 1 log cd s/m^2. (B, C) ERG changes in dark-adapted amplitudes of a-wave after non-flickering and flickering blue LED light exposure at -2 to 1.5 log cd s/m^2. (D) Comparison of a-wave changes of non-flickering and flickering blue LED light exposure at 0, 0.5, and 1 log cd s/m^2. (E, F) ERG changes in dark-adapted b-wave amplitudes after non-flickering and flickering blue LED light exposure at -2 to 1.5 log cd s/m^2. (G) Comparison of b-wave changes of non-flickering and flickering blue LED light exposure at 0, 0.5, and 1 log cd s/m^2. The amplitudes of the a- and b-waves reduced more significantly for the flickering blue LED light exposure group at 3 days than for the non-flickering group. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

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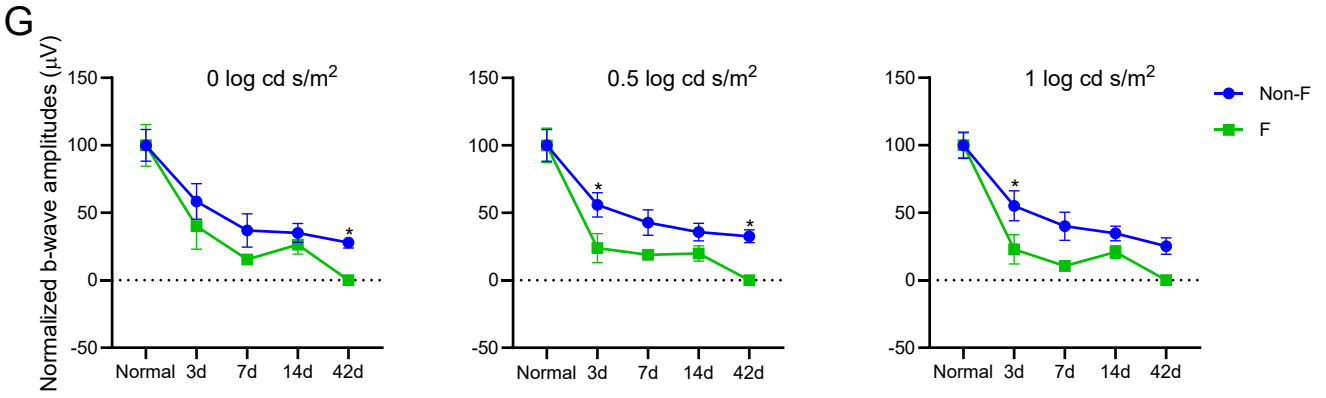

Fig. 2. Continued.

the standard before each experiment. The illuminance of the flickering blue light obtained using the LED lux meter was converted from 1500 lux for BALB/c mice and 1950 lux for C57BL/6J mice to 710 and 920 lux, respectively. For more accurate quantification of the flicker light output voltage, the flashing light can be calculated as a percentage flicker or flicker index (Fig. 1). The flicker stimulus generated by the blue led light in our apparatus had an average effective voltage of 20% with a duty cycle based on a flicker on and off interval of approximately 20 Hz [32].

The temperature during the exposure was maintained at 27.5±1.0°C. After exposure to LED light, the mice were kept in a dark room for 1 h and moved to climate-controlled conditions with 12-hour cycles of light and darkness.

ERG

ERG recordings were conducted according to the procedures described in our previous studies [12, 33]. The mice were kept in total darkness for 12 hours before ERG recording, and all the protocols were performed under low-intensity red light (λ>600 nm). The mice were anesthetized by intraperitoneal injections of 20 mg/kg zolazepam and 7.5 mg/kg xylazine. The mice were placed on a heating pad during the ERG recordings to maintain their body temperature. The corneas were covered with gold-ring contact electrodes. The ground electrode was subcutaneously positioned in the tail, and the reference electrode was placed in the ear. Short-term white flashes were administered as stimuli using a Ganzfeld stimulator (UTAS-3000; LKC Technologies, Gaithersburg, MD, USA). Under dark-adapted conditions, flash ERG recordings were obtained from both eyes, utilizing white light stimulation with intensities set at -2, -1.5, -1, -0.5, 0, 0.5, 1, and 1.5 log cd s/m^2 for scotopic ERG measurements. The mice were adapted in a photopic background with an intensity of 20 cd s/m^2 for 10 min. Stimulus intensities for photopic ERG were set at -0.5, 0, 0.5, 1, and 1.5 log cd s/m^2. Recordings were averaged five times. The scotopic and photopic ERG data values were obtained by averaging single responses recorded three times at 15-second intervals and five times at 3-second intervals, respectively. ERG data analysis was performed using UTAS software (EMWin, LKC Technologies, Gaithersburg, MD, USA) and GraphPad Prism 9.5.0 (GraphPad Software; San Diego, CA, USA).

SD-OCT

SD-OCT scanning was performed after the ERG recordings were completed to assess the correlation of structural changes with function in the same mice. First, the mice were anesthetized using a zolethyl/rompun cocktail (100/10 mg/kg), and both eyes were imaged using an SD-OCT ophthalmic imaging system (II Science, Busan, Korea) through pupils dilated with Mydrin P (Santen Pharmaceutical Co., Osaka, Japan). The corneas were treated with hydroxypropyl methylcellulose gel to prevent dehydration. For image acquisition, the mice were placed on a holding plate to stabilize fixation, and 27 B-scans were averaged to enhance the S/N ratio. SD-OCT images were obtained from cross-sectional en-face scans in normal mice and at 3, 7, 14, and 42 days after blue light exposure. The recordings were measured at several positions for each eye using commercial OCT software (II Science, Busan, Korea). Using this software, retinal thickness was evaluated for each eye. Eight equal distances (±150, ±300, ±450, ±600) in both the nasal and temporal directions from the optic nerve head were used for measurements.

Statistical analysis

Data are presented as mean±standard error of mean. All statistical analyses of ERG amplitude and retinal layer thickness were performed using GraphPad Prism 9.5.0 (GraphPad Software; San Diego, CA, USA) by two-way analysis of variance with Bonfer-

roni's multiple comparison test. Statistical significance was defined as $p<0.05$.

RESULTS

Functional changes in response to blue light exposure in albino mouse retina

Retinal damage was induced by excessive exposure to flickering and non-flickering blue light, and their functional changes were recorded and traced using ERG (Fig. 2). Fig. 2A shows the scotopic ERG response in normal and damaged mice at 3, 7, 14, and 42 days after non-flickering and 20-Hz flickering blue light exposure, respectively, as representative waveforms of a flash at 1 log cd s/m^2. The a-wave amplitudes of the ERG responses were significantly reduced 3 days after both blue light exposure ($p<0.05$) compared to those of the normal group (Fig. 2B, C). To compare the flickering and non-flickering groups more clearly, we normalized the a-wave amplitudes at 0, 0.5, and 1 log cd s/m^2 (Fig. 2D). The scotopic a-wave responses of the non-flickering blue light-induced group decreased abruptly on day 3 ($p<0.05$) followed by a minor increase or remained constant until day 42 ($p>0.05$). In contrast, the flickering blue light-induced damaged group showed different scotopic a-wave responses from those of the non-flickering group after 3 days, which was further reduced at 42 days ($p<0.05$). This

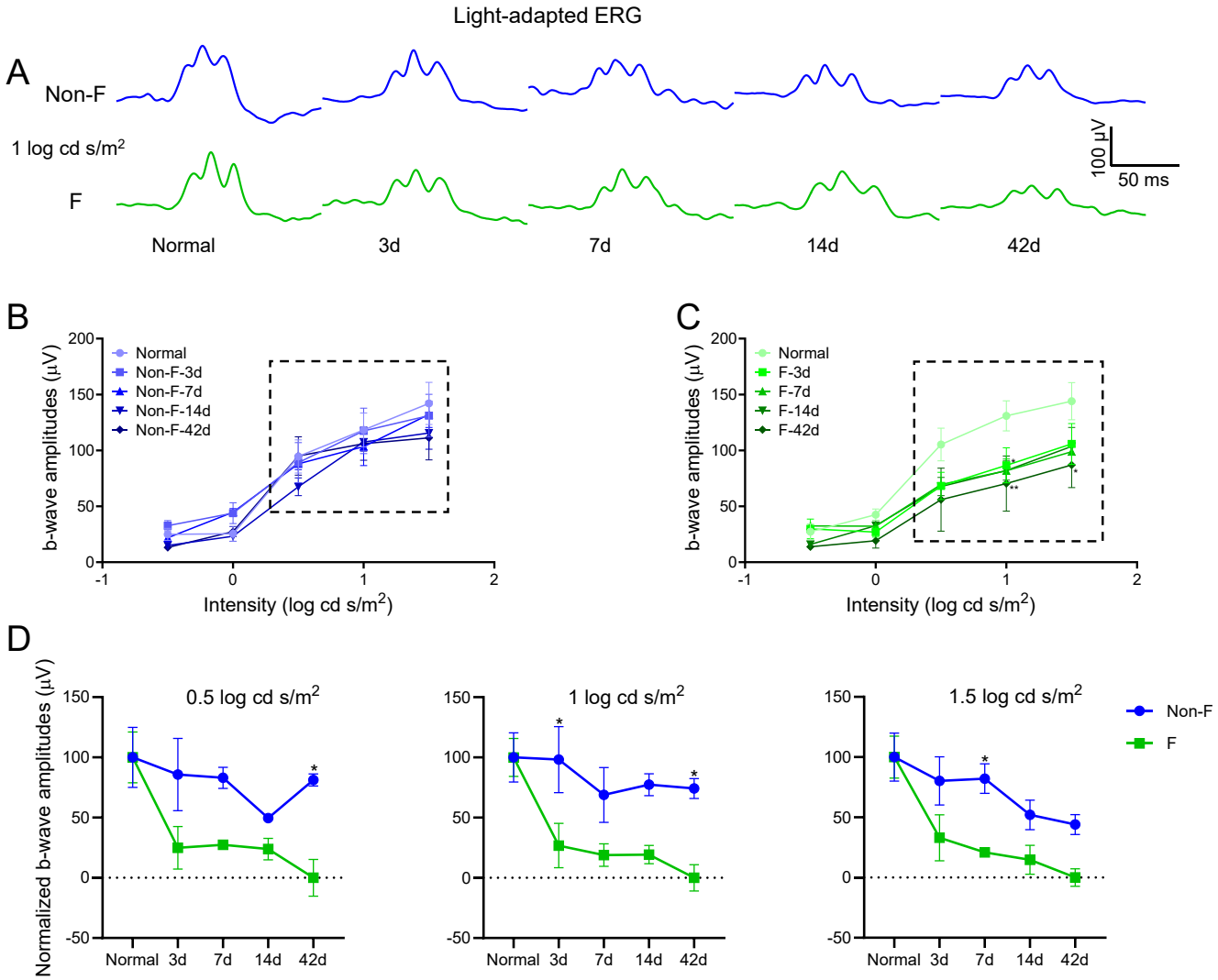

Fig. 3. Light-adapted b-wave amplitudes of BALB/c mice after blue light-emitting diode (LED) light exposure. (A) Representative electroretinography (ERG) waveforms before injury and at 3, 7, 14, and 42 days after blue LED exposure. The intensity of the stimulus flashes was 1 log cd s/m^2. (B, C) ERG changes in light-adapted b-wave amplitudes after non-flickering and flickering blue LED light exposure at -0.5~1.5 log cd s/m^2. (D) Comparison of b-wave changes after non-flickering and flickering blue LED light exposure at 0.5, 1, and 1.5 log cd s/m^2. The b-wave amplitudes significantly reduced more for the flickering blue LED light exposure group at 3 days than for the non-flickering group. *$p<0.05$; **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

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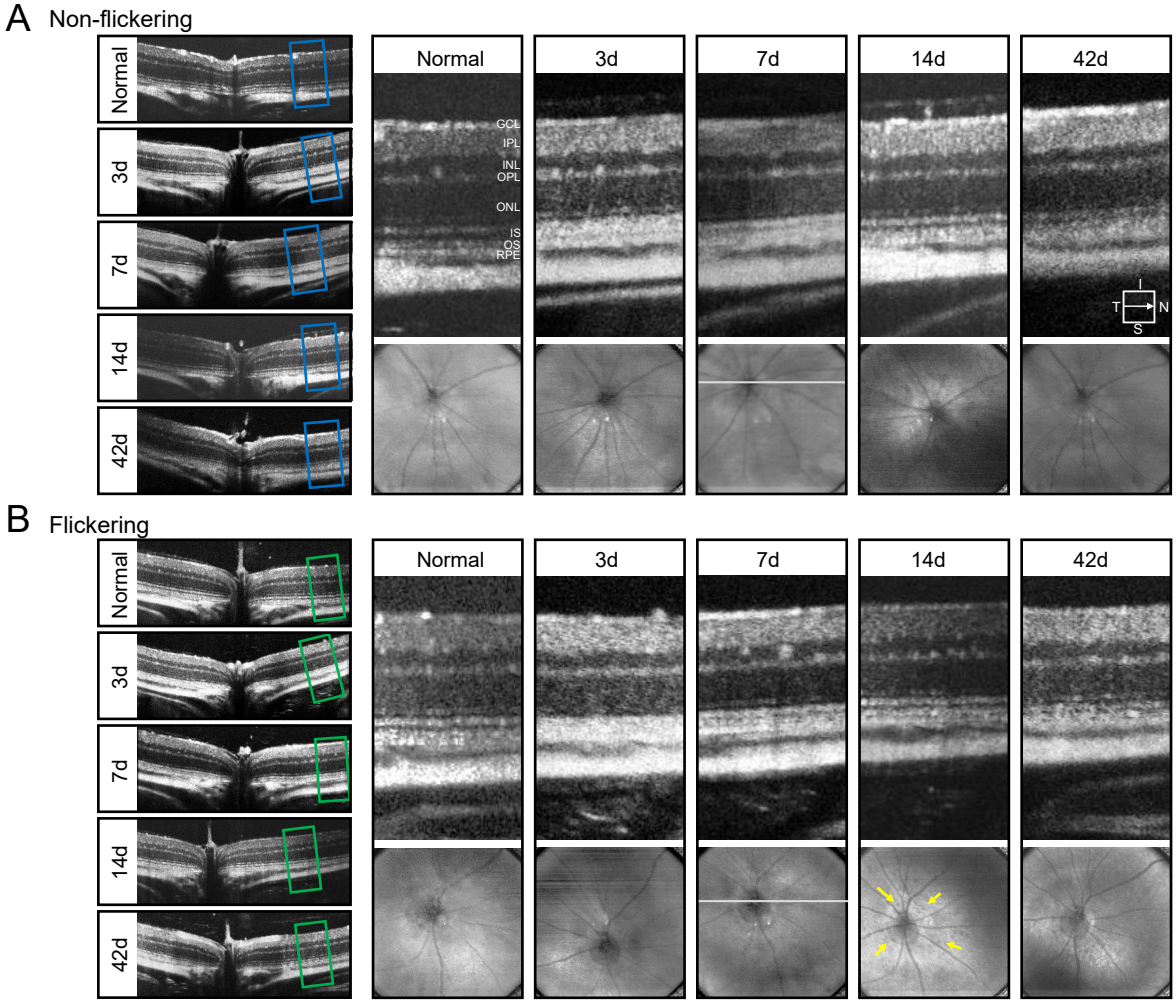

Fig. 4. Spectral-domain optical coherence tomography analysis of retinal structural changes in BALB/c mice exposed to blue non-flickering and flickering light-emitting diode (LED). (A, B) Changes in the en-face views and retinal layer thickness after non-flickering and flickering blue LED light exposures. Hyporeflective spots (yellow arrows) can be seen on en-face views of flickering blue light exposed at 14 days. (C, D) The retinal layer thickness was measured manually in normal and damaged mice at 3, 7, 14, and 42 days after blue light exposure. (E) Comparison of the changes in the thickness of the non-flickering and flickering blue LED light exposures. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium.

difference was statistically supported by normalized amplitudes at light intensities of 0, 0.5, and 1 log cd s/m^2 (p=0.0433, 0.0459 and 0.0086) (Fig. 2D). The difference in the scotopic a-wave responses between the flickering and non-flickering group were not statistically significant on day 3 and 7 (p>0.05), while it became significant on day 42 (p<0.05). According to the scotopic b-wave results, the flickering group showed a greater decrease than the non-flickering group starting from day 3 (p<0.05), and this difference persisted until the end of the 42-day experiment (p<0.05, Fig. 2E~G).

Photopic representative waveforms of flash at 1 log cd s/m^2 are shown in Fig. 3A. Regarding photopic amplitudes, only the b-wave results are presented (Fig. 3B~D) because BALB/c mice have nearly no photopic a-waves. The flickering blue light-induced damaged group amplitudes were significantly reduced at 3 days (p<0.05) and continued to decline until 42 days (p<0.05), while the photopic b-wave amplitudes of the non-flickering blue light-induced group only slightly decreased at 3 days (p>0.05) and thereafter demonstrated relatively small changes. The difference in photopic b-wave amplitudes between flickering and non-flickering groups always remained significantly different (p<0.05).

Taken together, excessive exposure of albino mice to 20-Hz flickering blue light resulted in a more severe functional decline than exposure to non-flickering blue light and had a more lasting damaging effect.

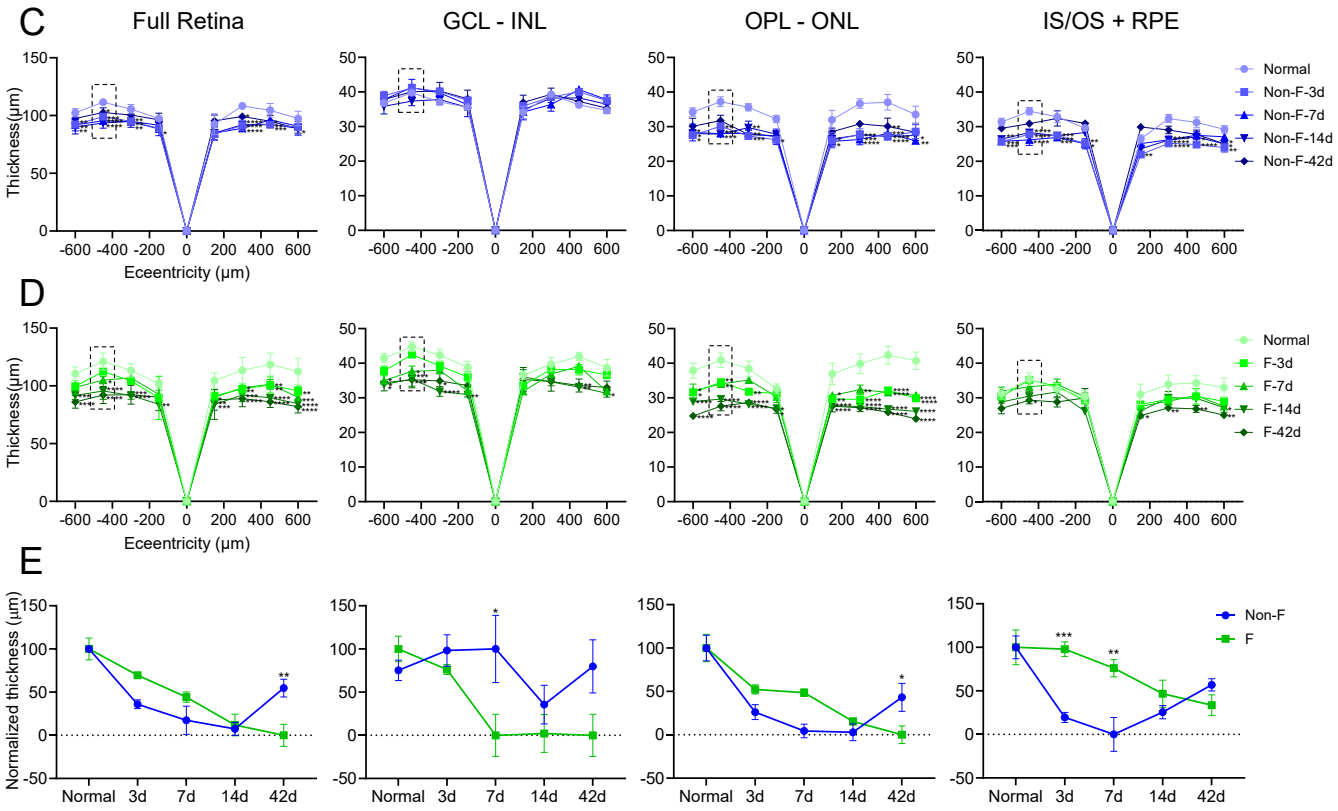

Fig. 4. Continued.

Structural changes in response to flickering blue light exposure in albino mouse retina

After a functional assessment of the damaged mice, we aimed to noninvasively investigate the structural changes of the retina. To accomplish this, OCT scanning was performed after the ERG recordings at each time point (Fig. 4). In normal mice, the retinal layers could be seen clearly. However, the layers of the retina became blurred and merged after both blue light exposures, especially in the outer layer of the retina (Fig. 4A, B). The retinal layer thickness was manually measured in normal and damaged mice at 3, 7, 14, and 42 days after blue light exposure (Fig. 4C, D). The en-face view of the flickering blue light exposure for 14 days revealed some hyporeflective spots (Fig. 4B, yellow arrows) of severe retinal damage. Normalized thickness data revealed distinct trends for the two groups (Fig. 4E). No significant changes were observed in the thickness of the inner layer in either group. The thickness of the outer retina gradually decreased after exposure to flickering blue light, whereas mice exposed to non-flickering blue light showed a decrease on day 3, which improved by day 42. Overall, the retina of the mice exposed to non-flickering blue light was initially thinner and thicker over time, indicating that the damage caused by non-flickering blue light was not very strong and could recover to some extent. However, the thickness of the flickering group continued to decrease, indicating that it caused more severe irreversible damage to the retina.

Effect of flickering blue light exposure on pigmented mouse retina

In C57BL/6J pigmented mice, which are relatively resistant to light stimulation, we determined whether flickering light caused more severe retinal damage on days 3 and 7 after excessive blue light exposure than non-flickering light. Both the a- and b-wave amplitudes of the ERG responses were significantly reduced 3 days after exposure to blue light (Fig. 5). However, the scotopic a- and b-wave and photopic b-wave amplitudes recovered after 7 days of exposure to non-flickering blue light (Fig. 5A, C, D, F, J, L), whereas the photopic a-wave amplitudes remained almost unchanged from the 3-day mark (Fig. 5G, I). However, the scotopic a- and b-wave amplitudes induced by the flickering blue light continued to decrease on day 7 (Fig. 5B, C, E, F, K, L). The photopic b-wave amplitudes demonstrated a similar trend.

The OCT results partially correlated with the ERG findings (Fig. 6). En-face views showed several hyperreflective spots on day 3 (Fig. 6B, yellow arrows), which further increased on day 7 after

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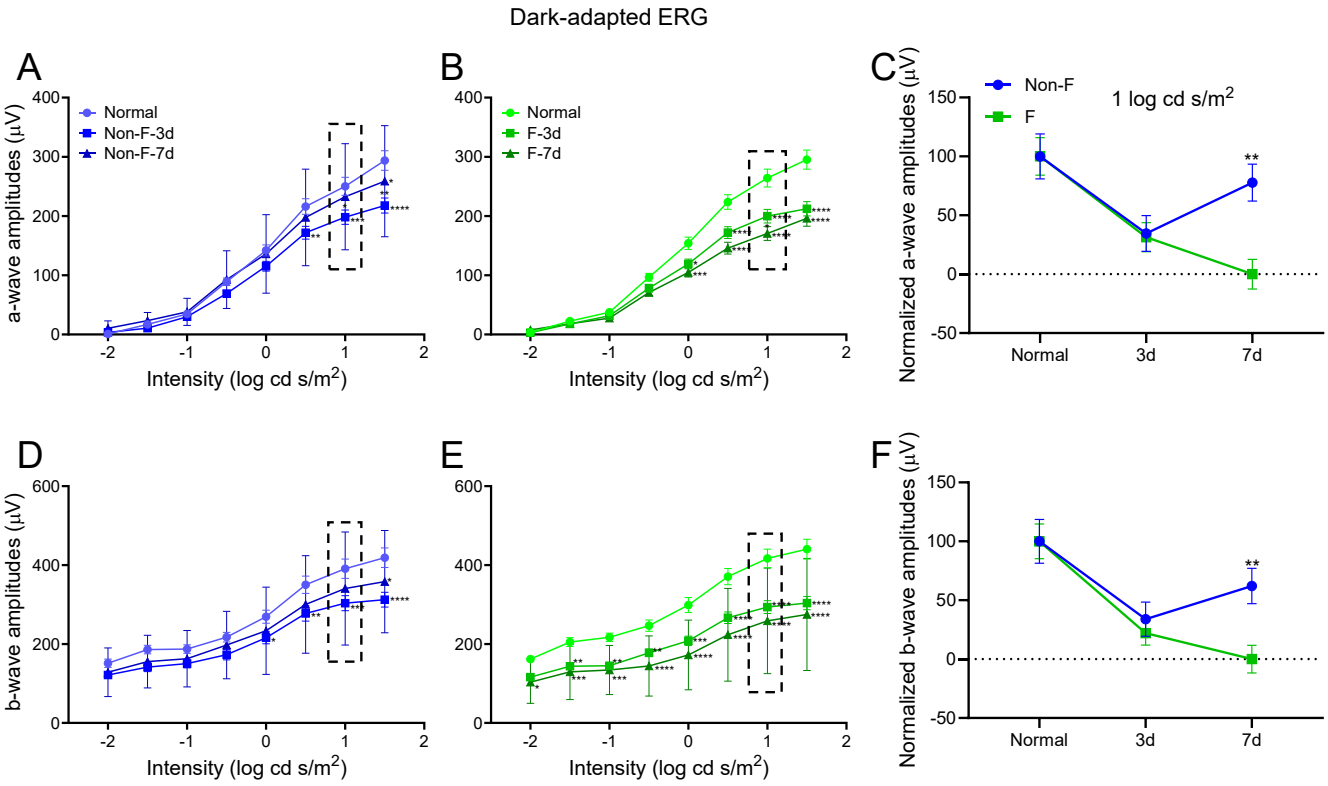

Fig. 5. Dark- and light-adapted amplitudes of a- and b-wave of C57BL/6J mice after blue light-emitting diode (LED) light exposure. (A, B, D, E) ERG changes in dark-adapted amplitudes of a- and b-waves after non-flickering and flickering blue LED light exposures at -2~1.5 log cd s/m^2. (C, F) Comparison of scotopic a- and b-wave changes after non-flickering and flickering blue LED light exposures at 1 log cd s/m^2. (G, H, J, K) ERG changes in light-adapted a- and b-wave amplitudes after non-flickering and flickering blue LED light exposures at -0.5~1.5 log cd s/m^2. (I, L) Comparison of the photopic a- and b-wave changes of the non-flickering and flickering blue LED light exposure at 1 log cd s/m^2. The amplitudes of the scotopic and photopic both a- and b-waves significantly reduced after flickering blue LED light exposure on day 7, while those for the non-flickering group increased a lot or remained. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

flickering blue light exposure. In contrast, the non-flickering group showed only a few hyperreflective spots on day 3. No significant changes were observed in the thickness of the full retinal or inner layers in either group. However, the inner retinal thickness decreased more for the flickering group than for the non-flickering group. Additionally, the thicknesses of the inner segment/outer segment and RPE transiently increased for the flickering group on day 3, which may have been due to the enlargement of the RPE during the early stage of blue light exposure [14].

After 7 days of damage, the retinal function and structure partially recovered with non-flickering blue light exposure. However, the group exposed to 20-Hz flickering blue light showed further deterioration in function and structure, which is sufficient to show that flickering light can cause more severe damage to the mouse retina.

DISCUSSION

We examined the effects of blue light on the retinas of mice. Steady blue light exposure induced RD in albino mice [12]. RD is accompanied by inflammation, microglial cell activation [10, 31], and oxidative stress [34]. This RD model is widely used to study the pathogenesis and treatment strategies of RD [35-37]. Consistent with the findings of our previous studies, this study demonstrated that RD was induced by flickering blue light exposure in pigmented mice, as well as in albino mice. Two noninvasive methodologies, ERG and OCT, were used to simultaneously observe alterations in retinal function and structure. In addition, flickering blue light caused significantly more severe damage to the retinal structure and function than non-flickering blue light, suggesting that flickering may be a risk factor for more severe retinal damage.

For flicker intensity, higher frequencies for the same amplitude difference were associated with higher average output amplitudes, approaching 100% duty cycle of non-flickering intensity (see

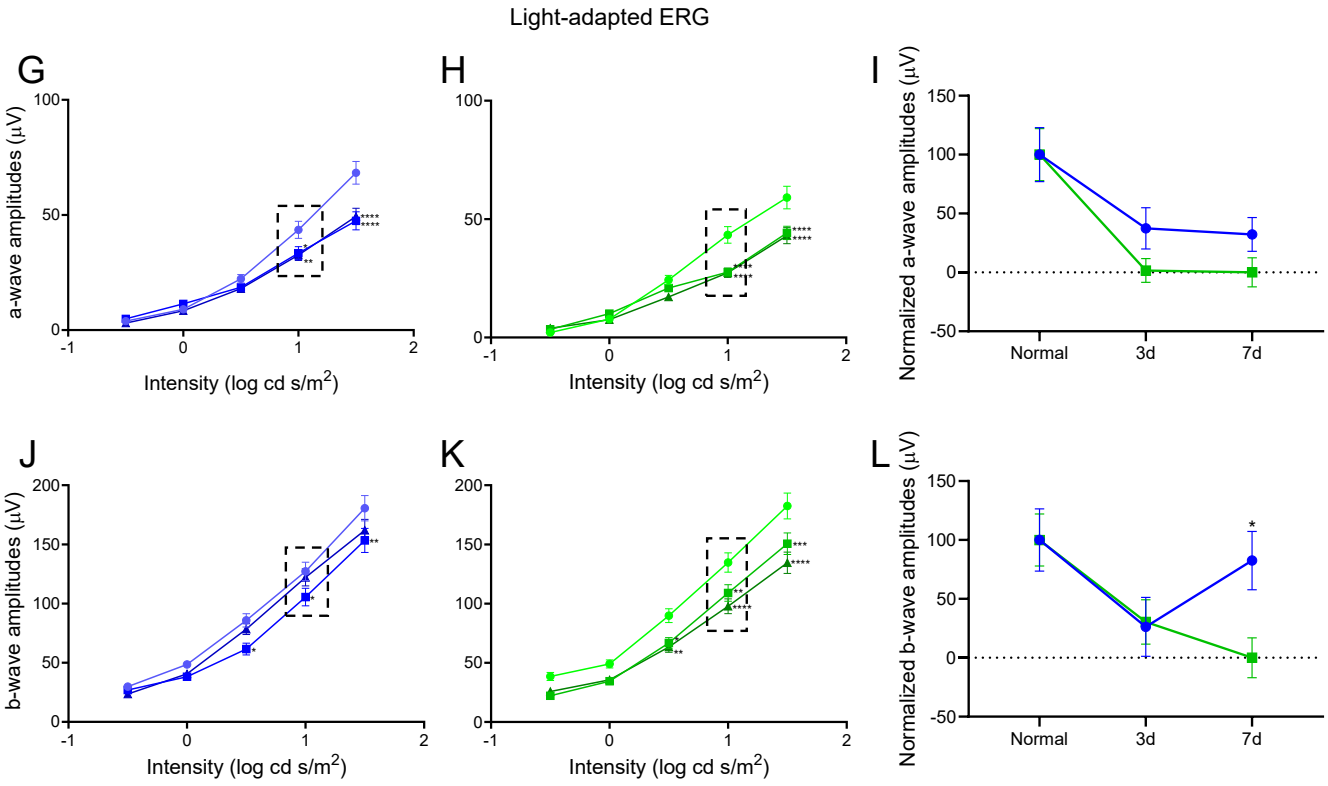

Fig. 5. Continued.

Methods) [32]. Consequently, the rapid flickering of light above the CFF value, which accounts for approximately 60% of our daily lighting, is an increasing concern because of the prolonged exposure of humans and other organisms [28]. To our knowledge, this study is the first to provide evidence that flickering at 20 Hz, which is below the typical range of 50~90 Hz (CFF in humans) [30], causes retinal damage in mice. Notably, the non-flickering blue light intensity used in this experiment was below the optimal level (1800 lux, as observed in our previous study [10]). Even at 20 Hz with an average luminance calculated to be only 20% of the non-flickering duty cycle, a reduction in retinal function was evident after blue light exposure. This suggested that flickering can be harmful to the retina. The greater reduction in the photopic ERG b-wave amplitude, which reflects cone-driven visual signals, is also noteworthy. This result can be explained by the relative suppression of rod photoreceptors and overstimulation of cones under exposure to very bright blue light at 20 Hz in mice adapted to darkness [38].

We found that BALB/c mice in the flicker group demonstrated similar patterns of damage over time in terms of retinal function and structure. However, the outer retinal thickness changes in the non-flicker group of BALB/c mice showed a slight recovery at day 42. Regarding ERG, the scotopic a-wave amplitude of non-flickering group at -1 log cd s/m^2 and photopic b-wave amplitude at 0.5 log cd s/m^2 (Fig. 3D) slightly increased on day 42, which was consistent with the OCT results to some extent. This can indicate that the function has been restored partly, but ERG only shows increase in a few intensities, which might be attributed to the fact that the retina has entered the recovery stage on 42d, but it is reflected structurally first. After structural signaling to be re-established, function maybe recover.

As a more susceptible subject to the flicker risk, we initially selected the albino mouse and subsequently monitored the effect in the C57BL/6J mice, for which several researchers have been unable to induce retinal damage with excessive white light alone [39, 40]. Functional and structural disruptions reached their maximum at 7 days following light damage even for cases of successful retinal damage induction with blue light exposure, after which there was gradual recovery [14]. These findings are consistent with our ERG results for C57BL/6J mice: rapid ERG recovery continued after 7 days of light-induced damage until day 42. Due to inter-individual variations and other uncontrollable factors affecting ERG amplitude, we could not identify statistically significant differences between the functional changes in the two groups after 14 days. Nevertheless, the functional decline caused by flickering blue light in the C57BL/6J mice group was consistent with the results ob-

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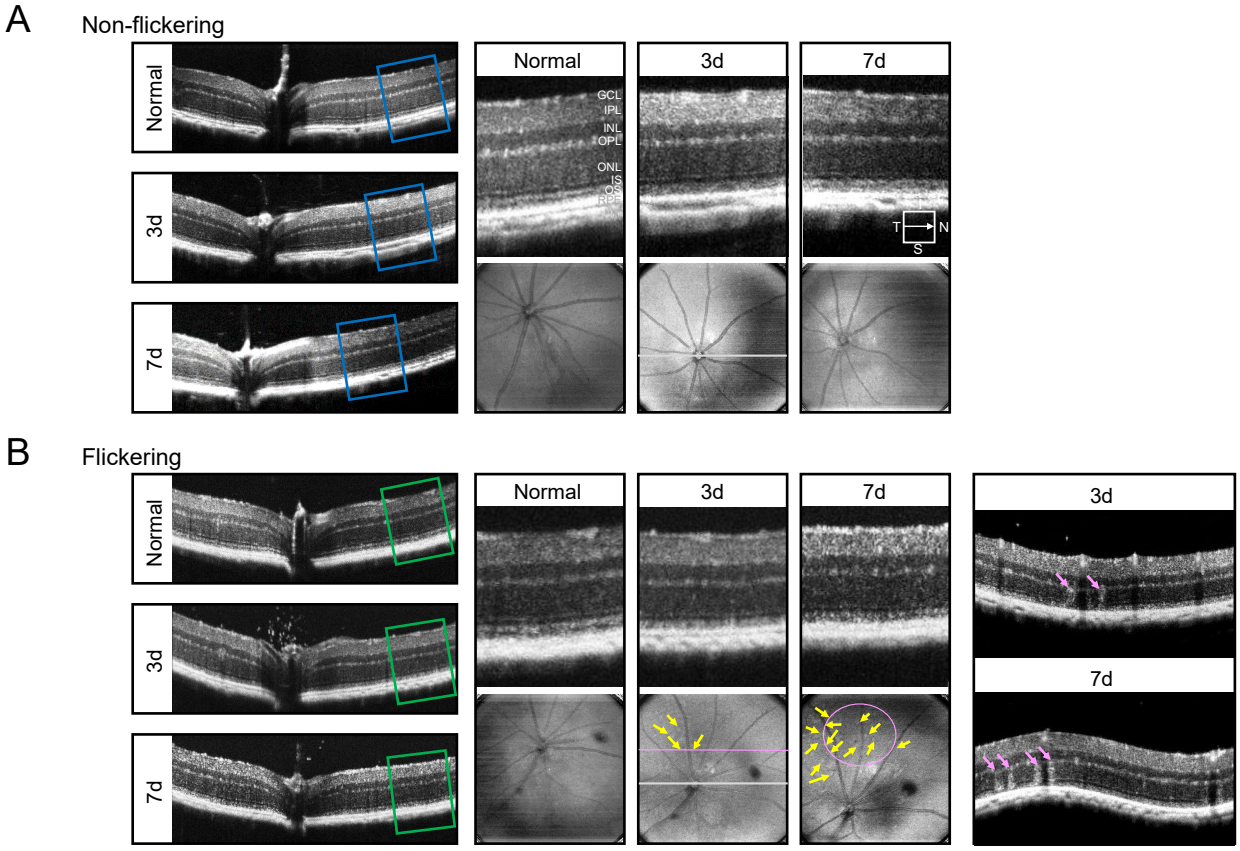

Fig. 6. Optical coherence tomography analysis of retinal structural changes in pigmented mice (C57BL/6J) exposed to blue light-emitting diodes (LEDs): Non-Flickering vs. Flickering. (A, B) Changes in the en-face views and retinal layer thickness after non-flickering and flickering blue LED light exposures. Hyperreflective spots (yellow arrows) can be observed on en-face views at 3 and 7 days after flickering blue light exposure. Examples of hyperreflective abnormalities in the outer retinal layers (pink arrows) obtained from two additional B-scans (horizontal and circular pink lines) at 3 and 7 days after flickering blue light exposure are shown separately on the right. (C, D) The retinal layer thickness was measured manually in normal and RD mice at 3 and 7 days after blue light exposure. (E) Comparison of changes in thickness after non-flickering and flickering blue LED light exposure. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; OS, outer segment; RPE, retinal pigment epithelium.

tained in the BALB/c group for at least up to 7 days. Furthermore, en-face OCT revealed an increase in the hyperreflective spots after day 7 (data not shown). This finding is consistent with previous studies that identified hyperreflective spots due to cellular abnormalities in the RPE and/or photoreceptors [41]. Further studies of the cellular and molecular levels via TUNEL staining, immunohistochemical analysis, and RT-PCR, among others, are needed to investigate the reason why flickering light causes more severe retinal damage than non-flickering steady light.

Exposure to the blue light emitted by smartphones can lead to chronic light-induced retinal damage in humans [42]. Additionally, flickering light has a stronger inhibitory effect on melatonin secretion than non-flickering light [43, 44]. The health effects of flickering on humans can be divided into immediate (such as epileptic seizures, discomfort, headaches, and functional impairment) and long-term (including persistent discomfort, headaches, and visual impairment) [27]. The biological impact of flickering on humans depends on factors such as flicker frequency, brightness, and duration of exposure. The retina is the sensory organ most directly affected by light stimuli. Therefore, it is important to determine the characteristics of flickering light that may contribute to retinal damage.

In conclusion, we demonstrated that 20-Hz flickering blue LED light can induce more persistent and severe retinal damage than non-flickering blue light and can be used as a novel model for RD. Furthermore, the present study suggests that unsteady flickering blue light is more likely to cause blue-light-induced damage, even at frequencies as low as CFF. The effect of flickering light obtained in this study and its non-invasive functional structural assessment can be extended to mouse RD model studies to elucidate the association of different frequencies of blue light stimulation with AMD progression and the underlying disease mechanisms.

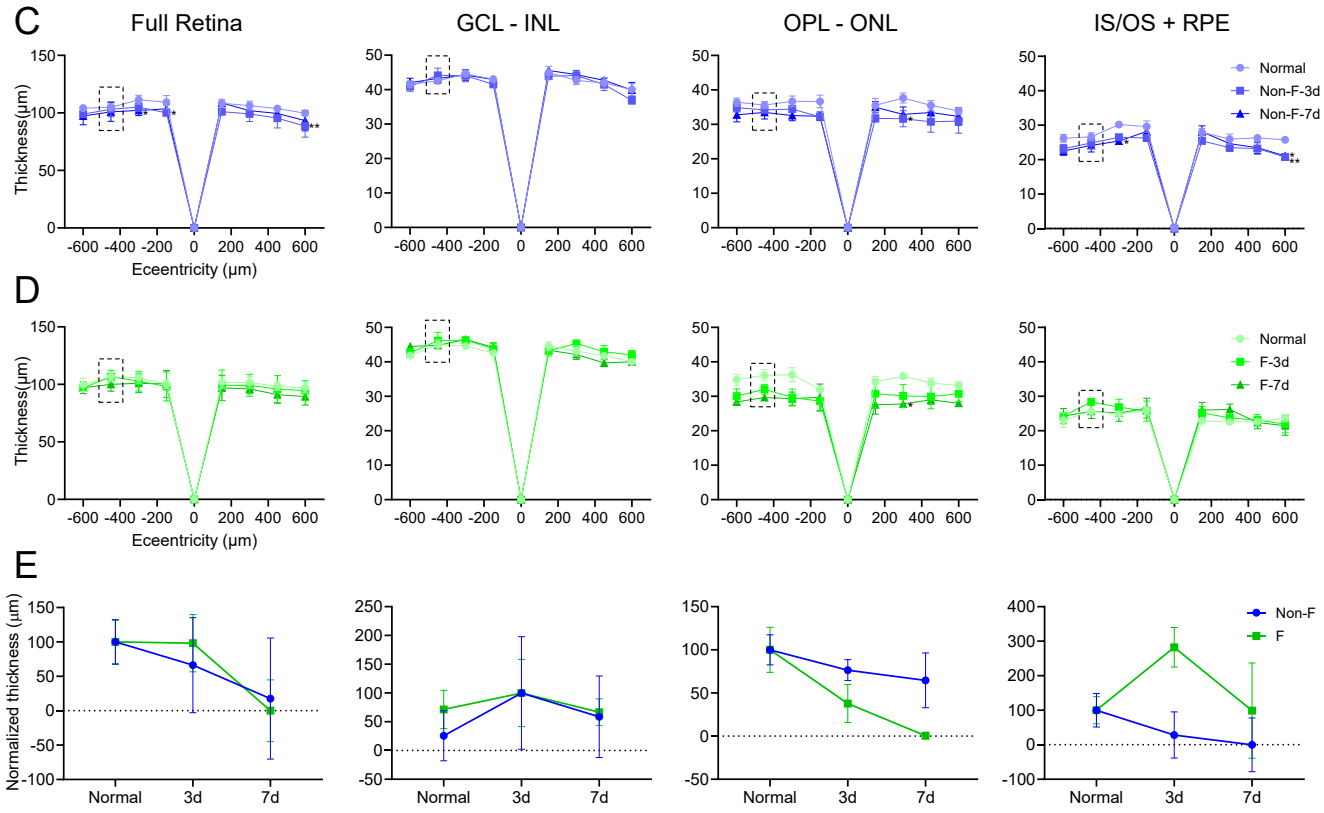

Fig. 6. Continued.

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