Effects of artificial light with different spectral composition on eye axial growth in juvenile guinea pigs

Xinyu Xu, Jiayu Shi, Chuanwei Zhang, Lixin Shi, Yujie Bai, Wei Shi, Yuliang Wang

Department of Ophthalmology, Affiliated hospital of Nanjing University of Chinese Medicine, Nanjing, China

ABSTRACT

The purpose of the study was to investigate the effect of artificial light with different spectral composition and distribution on axial growth in guinea pigs. Three-week-old guinea pigs were randomly assigned to groups exposed to natural light, low color temperature light-emitting diode (LED) light, two full spectrum artificial lights (E light and Julia light) and blue light filtered light with the same intensity. Axial lengths of guinea pigs' eyes were measured by A-scan ultrasonography prior to the experiment and every 2 weeks during the experiment. After light exposure for 12 weeks, retinal dopamine (DA), dihydroxy-phenylacetic acid (DOPAC) levels and DOPAC/DA ratio were analyzed by high-pressure liquid chromatography electrochemical detection and retinal histological structure was observed. Retinal melanopsin expression was detected using Western blot and immunohistochemistry. After exposed to different kinds of light with different spectrum for 4 weeks, the axial lengths of guinea pigs' eyes in LED group and Julia light group were significantly longer than those of natural light group. After 6 weeks, the axial lengths in LED light group were significantly longer than those of E light group and blue light filtered group. The difference between axial lengths in E light group and Julia light group showed statistical significance after 8 weeks ($p<0.05$). After 12 weeks of light exposure, the comparison of retinal DOPAC/DA ratio and melanopsin expression in each group was consistent with that of axial length. In guinea pigs, continuous full spectrum artificial light with no peak or valley can inhibit axial elongation *via* retinal dopaminergic and melanopsin system.

Key words: Artificial light; spectral composition; axial growth; guinea pigs; dopamine.

Correspondence: Yuliang Wang, Department of Ophthalmology, Affiliated hospital of Nanjing University of Chinese Medicine, 155 Hanzhong Road, Qinhuai District, Nanjing 210029, Jiangsu Province, China. E-mail: wyl0601@126.com

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Introduction

With the soaring prevalence of myopia all over the world, especially in Asia, more attention has been drawn to this issue. Numerous studies have shown that time spend outdoors is a strong protective factor against myopia, though the exact dose-response relationship is still unknown.[1-4] A striking difference between outdoor and indoor environment is light. Artificial light is quite different from natural light in terms of illuminance, rhythm, stroboscopic and spectrum.[5] A study by Prepas[6] suggests that the increase in myopia incidence may be related to the emergence of artificial light sources.

Previous animal studies demonstrated that different monochromatic lights are closely related to refractive development. When raised in red light, rhesus monkeys and tree shrews became consistently more hyperopic, while exposed to short-wavelength light they tended to become more myopic.[7-9] However, chickens became more myopic in red and more hyperopic in blue light, as well as fish and guinea pigs.[10-13] Nowadays the artificial light environment around us is mostly polychromatic light composed of long, medium and short wavelengths of various monochromatic light, which is different from lights mentioned in the above experiments. Natural light has continuous spectrum with no peak or valley, while light-emitting diode (LED) light, the most commonly used artificial light, has 400-460 nm wavelength blue light peak as well as 480 nm wavelength valley. Whether the difference of light spectral composition may have an impact on myopia development drew our attention. Few studies about whether imitated natural light affect refraction development were reported at present. Therefore, we attempt to design full spectrum light which resembled the spectral composition and distribution of natural light to study on New Zealand rabbits, and found that full spectrum light has protective effect on axial elongation and retinal structure damage.[14] Besides, benefits of blue-light filtering intraocular lens on eye health have been reported.[15] In this study, we choose the most commonly used low color temperature LED light, two full spectrum lights which resembled the spectral composition of natural light and blue light filtered light to study on guinea pigs aiming to explore the effect of different spectrum based-artificial lights on axial growth, and whether full spectrum based-artificial light can reduce the light-induced damage on refractive development.

Materials and Methods

Animals and experimental design

Thirty three-week-old guinea pigs, obtained by Laifu Animal Experimental Centre of Jiangsu Province China, were raised in a homothermal room with temperature of 18-24°C and relative humidity of 55% to 65%. We make sure all guinea pigs have free access to sufficient and fresh food and water. The treatment and care of the animals were in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Our animal research was approved by the Animal Care and Ethics Committee at Affiliated hospital of Nanjing University of Chinese medicine, Jiangsu, China. In order to investigate the protective effect of natural light spectrum based-LED light on refractive development, four different spectral composition lights with same light intensity were applied in the study. Accordingly, all guinea pigs were randomly assigned to one of the following subgroups, 6 for each group: Natural light group, LED group (low color temperature LED light), E light group, Julia light group and blue light filtered light group (see below).

Lighting

Guinea pigs were kept in cages covered with black cloth and with lights on top of the cage. Photo-sensory and self-adjusting lighting device was placed outside the cage in order to automatically adjust the illumination intensity according to outside light intensity so as to ensure the luminous rhythm in the cage was synchronous to the natural light. The average light illuminance was determined 350 lux in each group, and we adjust the distance between cage and window in order to reach such illuminance value in consideration of the weather. We use frosted glass to make the window side of the cage. All optical parameters including light spectral composition, illuminance and flicker frequency were detected with a fluorospectrophotometer (HR2000; Ocean Optics, Inc., Osaka, Japan; detection limit is 200-1100 nm).

Four different artificial lights were applied in this experiment. The most commonly used low color temperature LED light which emit discontinuous spectrum with a blue light peak at wavelength of 450 nm was selected. Besides, we chose three kinds of continuous spectrum based artificial light: i) E light: imitated natural light spectral composition with continuous wavelengths ranging from approximately 390 to 780 nm; ii) Julia light (J light): the spectrum profile was similar to E light, except for a small peak at 430nm followed by a small valley at 450nm; iii) blue light filtered light (B light): continuous spectrum with wavelength of 400nm below filtered (Figure 1).

Ocular biometry

Axial lengths of guinea pigs' eyes in each group were measured before the experiment and every 2 weeks during the experiment. All guinea pigs were measured at 8:00 a.m. without lid retractors. We use A-scan ultrasonography with a 10-MHz probe to detect the axial length (KN-1800; Kangning Medical Device Co., Ltd., Wuxi, Jiangsu Province, China). Before the measurement, one drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Geneva, Switzerland) was administered to the eye. We make sure the ultrasound probe directly contacts the corneal apex, and pay special attention to ensure the probe was vertical to the corneal surface. Results from 10 readings were averaged for each eye measured.

High-pressure liquid chromatography-electrochemical detection

All guinea pigs were sacrificed after light exposure for 12 weeks, retinal dopamine (DA) and dihydroxy-phenylacetic acid (DOPAC) levels were analyzed by high-pressure liquid chromatography electrochemical detection. The whole retina from each eye were carefully isolated on ice under a microscope, immediately frozen on dry ice, and stored at -80°C. Each retina sample was homogenized in phosphate buffered saline (PBS) with 0.1 mM ethylene diamine tetraacetic acid (EDTA) and then centrifuged at 6000 rpm for 10 min at 4°C. The supernatants were mixed with an equal volume of perchloric acid and then centrifuged at 20,000 rpm for 10 min at 4°C. The supernatants were collected for further analysis. High-pressure liquid chromatography-electrochemical detection was conducted at the Tangzhongying Laboratory at Nanjing University of Chinese Medicine, Jiangsu. The conductors were blinded to the experimental groupings. The HPLC system (Eclipse Plus C18, 2.1*150 mm, 3.5 μm) was run with a test mobile phase containing 100 mM NaH_2PO_4, 50 mM citric acid buffer (pH 3.0, adjusted with sodium hydroxide), 200 mg/L sodium 1-octanesulfonate, 10% methanol, and 50 μM EDTA at a flow rate of 0.2 mL/min. Twenty microliters sample of each group were injected onto the column. The data were analyzed by the Chromeleon 7 chromatography data system software (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Retinal DA and

DOPAC levels were compared between groups, as well as the ratio of DOPAC/DA as an indicator of DA turnover of the guinea pigs' eye.

Western blot analysis

Western blot analysis was performed as we previously described.[16] In brief, 25 mg of protein lysate were loaded onto 10% SDS-polyacrylamide gels (SDS-PAGE) for electrophoresis. After transferred to polyvinylidene difluoride membrane (Millipore, IPVH00010, USA), the samples were blocked in 5% skim milk in TBST for 2 h at room temperature. The membrane was then incubated with primary antibodies against melanopsin and GAPDH (1:1000, Abcam Technology, Waltham, MA, USA) overnight, followed by the secondary antibodies (1:5000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) incubation for 1 h at 4°C. The membranes were visualized by enhanced chemiluminescence reagents (WBKLS0100; Millipore, Burlington, MA, USA). Melanopsin expression level was quantified using ImageJ software (National Institute of Health, Bethesda, MD, USA) and normalized to GAPDH.

Retinal histopathological observation

The retinal sample were put into stationary liquid containing 4% paraformaldehyde for more than 24 h, dehydrated in 80%, 90%, 95% and 100% ethanol for 2 h each, placed in 65°C for 3 h into melting paraffin, embedded, and 4 μm serial sections were cut at -20°C. Sections were then stained with hematoxylin and eosin (H&E). The histopathological morphology of retinal structure, including pigment epithelium layer, photoreceptor layer and nerve fiber layer, were observed under microscope (Leica DMR, Leica Microsystems Inc., Wetzlar, Germany).

Immunohistochemistry

Immunohistochemistry was conducted to identify the location and expression of melanopsin. The retinal neuroepithelium tissue was fixed in 4% paraformaldehyde for more than 24 h, then trimmed with a scalpel, placed in the dehydration box with the corresponding label. After dehydrated in 75% ethanol for 4 h, 85% ethanol for 2 h, 90% ethanol for 2 h, 95% ethanol for 1 h, absolute ethanol for 30 min (two changes), , ethanol-benzene for 5-10 min, xylene for 5-10 min (two changes), the tissues were then put in 65°C for 3 h to melting paraffin. Tissues were embedded and 4 μm serial sections were cut at -20°C. After drying for 2 h at room temperature, sections were rinsed three times in 0.01 M PBS and then incubated in 0.4% Triton X-100 (Sigma-ldrich, St. Louis, Missouri, USA) for 10 min. After rinsing three times in 0.01 M PBS, sections were incubated in 10% normal goat serum (Sigma-Aldrich, St. Louis, Mi, USA) for 30 min at 37°C. Primary anti-melanopsin antibody (1:200, AB19306; Abcam, Cambridge, UK) was incubated with the sections overnight at 4°C in a humidified chamber. After rinsing three times in PBS, the HRP-labeled secondary antibody goat anti-rabbit IgG (1:100, AP132R, Chemicon International Inc., Temecula, CA, USA) was applied for 2 h at room temperature. Immunolabeled sections were rinsed three times in PBS, mounted on slides with phosphoglycerol, and observed under a microscope (Leica DMR). The immunopositive cells' density was assessed by counting the number of immunopositive cell per 100 cells.

Statistical Analysis

The results are presented as mean ± standard deviation (SD). All the statistical analysis was conducted using SPSS 22.0 (SPSS, Chicago, IL, USA). Data were collected from both eyes of each guinea pig, and results from right and left eyes were averaged for each guinea pig. For the comparison of two independent groups, an unpaired *t*-test was used. One-way analysis of variance (ANOVA) was used for comparisons of the difference among multiple groups, followed by *post-hoc* tests using Tukey's honestly significant difference (HSD). The limit of significance was set at two-tailed 0.05.

Results

Effect of different light exposure on axial lengths

Before the experiment, the difference between baseline axial lengths of guinea pigs' eyes in different groups has no statistical significance ($p>0.05$). As exposed to five kinds of light with different spectrum, the axial length of guinea pigs' eyes in each group gradually increased throughout the experiment. After 4 weeks, the axial lengths of guinea pigs' eyes in L group (LED group) and J light group (Julia light group) were significantly longer than those of N group (natural light group) ($p<0.05$). After 6 weeks, the axial lengths of guinea pigs' eyes in L light group were significantly longer than those of E light group and B light group (blue light filtered group) ($p<0.05$). The difference between axial lengths in E light group and J light group showed statistical significance for 8 weeks ($p<0.05$). The above differences all showed statistical significance until 12 weeks of light exposure. The difference between axial lengths of other groups at other time points showed no statistical significance (Table 1).

Table 1. Comparison of axial lengths of guinea pigs' eyes upon different spectrum light exposure at specific time (mm).

Time	Natural light group	LED light group	E light group	J light group	B light group
0 week	7.59±0.10	7.52±0.11	7.54±0.15	7.53±0.11	7.56±0.09
2 weeks	7.63±0.11	7.71±0.09	7.66±0.13	7.71±0.10	7.71±0.10
4 weeks	7.71±0.14	7.94±0.16*	7.78±0.13	7.91±0.14*	7.79±0.15
6 weeks	7.90±0.21	8.28±0.14**	7.98±0.17$^{\#}$	8.11±0.16*	8.05±0.12$^{\#}$
8 weeks	7.97±0.16	8.39±0.13**	8.02±0.15$^{\#\#}$	8.25±0.13**	8.11±0.16$^{\#}$
10 weeks	8.11±0.16	8.47±0.13**	8.16±0.14$^{\#\#}$	8.35±0.14*	8.24±0.15$^{\#}$
12 weeks	8.19±0.15	8.60±0.15**	8.24±0.16$^{\#\#}$	8.43±0.16**	8.31±0.13$^{\#\#}$

J light group, Julia light group; B light group, blue light filtered group; *$p<0.05$, **$p<0.01$ compared with natural light group at the same time point; $^{\#}p<0.05$, $^{\#\#}p<0.01$compared with LED light group at the same time point.

Retinal DA, DOPAC levels and DA turnover

After 12 weeks of light exposure, retinal DA levels of guinea pigs' eyes were 10.06±1.75 ng/g, 3.04±0.89 ng/g, 5.94±1.51 ng/g, 4.47±1.09 ng/g and 5.51±1.13 ng/g in N group, L group, E light group, J light group and B light group, respectively. In addition, retinal DOPAC levels of guinea pigs' eyes were 12.17±1.89 ng/g, 3.10±1.55 ng/g, 7.01±1.80 ng/g, 4.74±1.92 ng/g and 6.39±1.84 ng/g in N group, L group, E light group, J light group and B light group, respectively. Retinal DA and DOPAC level in natural light group was significantly higher than those of other 4 groups, and those in L group was dramatically lower than those in E light group and B light group ($p<0.05$). As we known, the calculated retinal DOPAC/DA ratio is a measure of DA turnover in the corresponding tissue. After 12 weeks of light exposure, retinal DOPAC/DA ratio in natural light group was significantly higher than those of L group and J light group, while that in L light group was significantly lower than those of E light group and B light group and that of E light group was apparently higher than that of J light group ($p<0.05$), which was consistent with axial lengths outcomes (Figure 2).

Histopathological morphology of retinal structure

No remarkable pathological change was observed in the retinal specimen of natural light group (Figure 3A). In artificial light group, strong edema was prominent in the ganglion cells. We observed degenerative changes in the ganglion cells, vacuolated spaces particularly in the inner nuclear and the ganglion cell layers (Figure 3 B-D).

Melanopsin expression in retina

Melanopsin was detected by immunohistochemistry in all cases. Melanopsin-immunolabeled cells were found in the retinal ganglion cell layer of each group. Labeled cells were more densely distributed in the retinal ganglion cell layer of eyes in natural light group,E light group and B light group, compared with eyes of either L light group or J light group. The number of stained cells in the eyes of natural light group showed no statistical significance compared to that of E light group and B light group after 12 weeks of light exposure (Figure 4). Western blot analysis (Figure 5) indicated that retinal melanopsin protein expression in L light group and J light group were significantly lower compared with that of natural light group ($p<0.05$), and those of E light group and B light group were markedly higher than that of L group ($p<0.05$), while those of E light group and B light group showed no statistical significance compared with that of natural light group ($p>0.05$).

Discussion

Nowadays, the effect of light spectral composition on eye growth and refractive development have been studied in a number of animal models.[17,18] Gawne *et al.* demonstrated that the infant

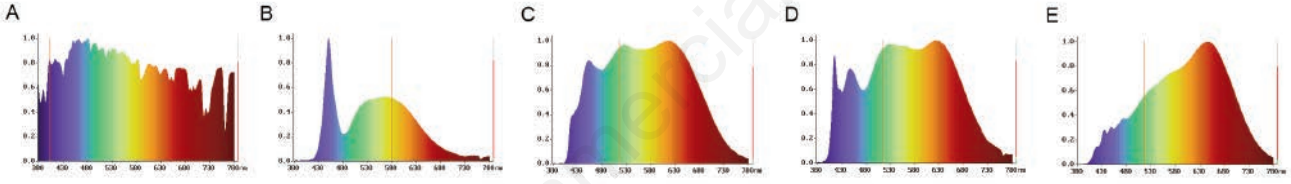

Figure 1. The spectral composition and distribution of natural light and four artificial lights. A) Natural light. B) Low color temperature LED light. C) E light. D) Julia light. E) Blue light filtered light.

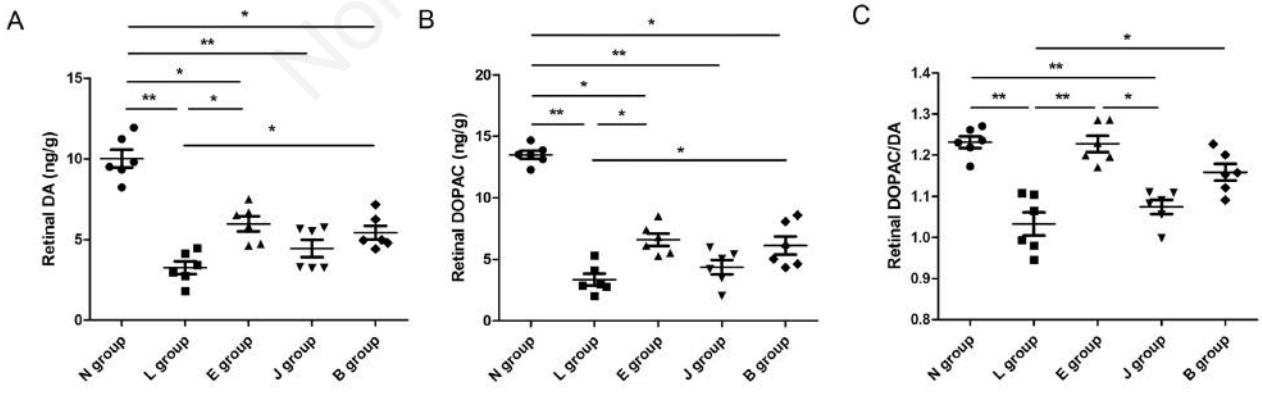

Figure 2. Concentrations of: (A) retinal dopamine (DA), (B) dihydroxy-phenylacetic acid (DOPAC), and (C) DOPAC/DA ratio in different groups after light exposure for 12 weeks. Concentrations of retinal DA (A), DOPAC (B) in natural light group was significantly higher than those of other 4 groups, and that of LED light group was dramatically lower than those of E light group and blue light filtered group. Retinal DOPAC/DA ratio in N group was significantly higher than those of LED light group and J light group, while that in LED light group was significantly lower than those of E light group and blue light filtered group, that of E light group was apparently higher than that of J light group. N group, natural light group; L group, LED light group; E group, E light group; J group, Julia light group; B group, blue light filtered group; *p<0.05, **p<0.01.

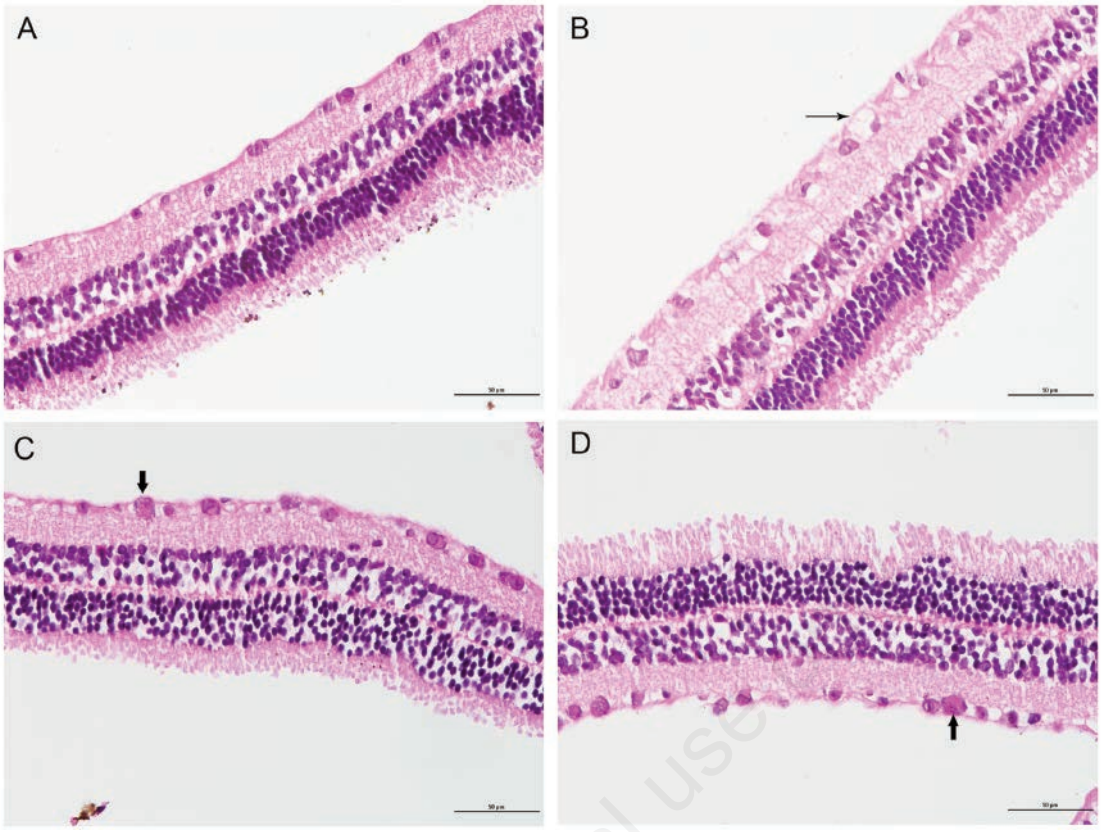

Figure 3. Artificial light-induced degenerative changes of retinal structure were observed. A) Natural light group. B) LED light group. C) Julia light group. D) Blue light filtered group. Degeneration in the ganglion cell layer, prominent edema in the ganglion cell layer (short arrow), vacuolization in the inner nuclear and ganglion cell layer (long arrow) are representative of changes seen in artificial light groups. Hematoxylin and eosin, ×400.

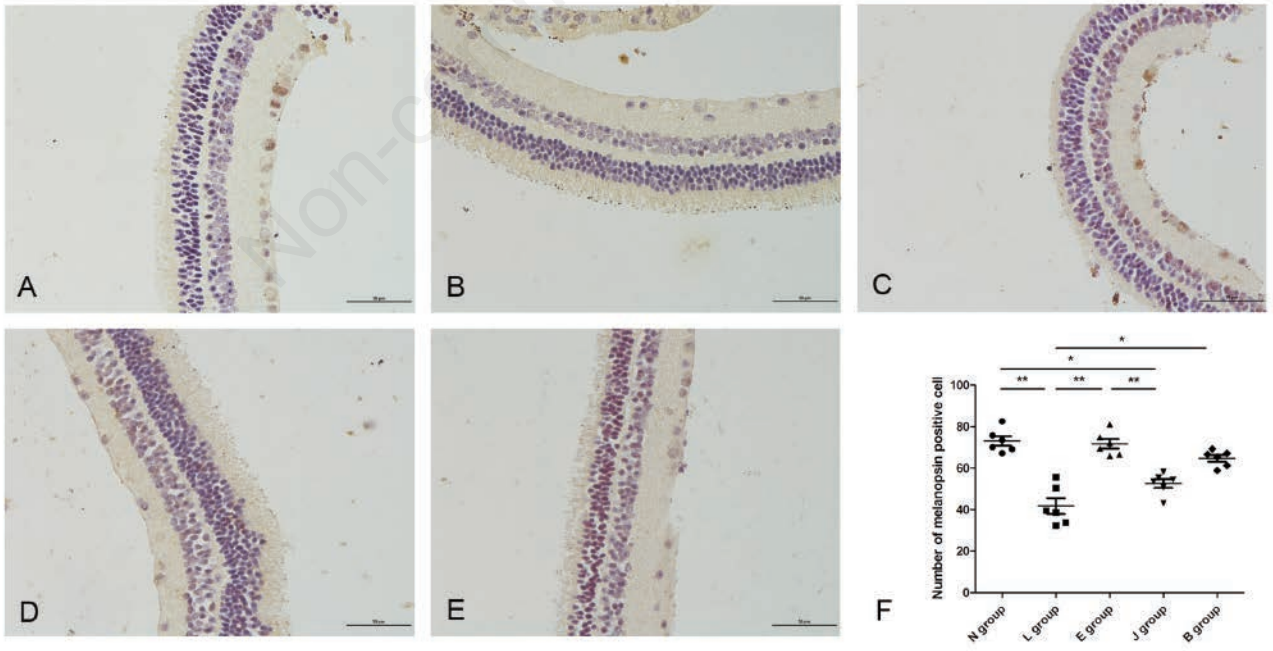

Figure 4. Photomicrographs showing melanopsin-immunolabeled cells in the retinal ganglion cell layer of guinea pigs exposed to different light for 12 weeks. Melanopsin-positive cells are stained brown. Stained cells were more densely distributed and more intensively stained in the retinal ganglion cell and inner nuclear layers of eyes in natural light group, E light group and blue light filtered group, compared with eyes of either in LED light group or J light group. A) Natural light group. B) LED light group. C) E light group. D) Julia light group. E) Blue light filtered group. F) Quantification of melanopsin-positive cells. *p<0.05, **p<0.01.

tree shrews exposed to red light (626±10 nm) were more hyperopic compared with those raised in white fluorescent lighting, so were older juvenile and adolescent tree shrews.[8,19] The infant monkeys wearing long wavelength pass (red) filters (wavelengths longer than 660 nm) were induced significantly hyperopic shift than those wearing neutral density filters and those under typical indoor lighting.[7] Hung *et al.* reported that narrow-band long wavelength lighting not only resulted in axial hyperopia, but also inhibited axial elongation induced either by form deprivation or hyperopic defocus.[20] Chickens became more myopic in red and more hyperopic in blue light, and so were fish and guinea pigs. Why manipulations of the spectral composition have opposite effects in different animal models is an open question. At present, most of the research about light spectrum focused on monochromatic light, and little on mixed light formed by multi-spectral components. We selected the most commonly used artificial light- low color temperature LED light and three kinds of continuous spectrum based-LED light to explore whether imitated natural light has protective effect against axial elongation caused by artificial light.

Guinea pigs are widely used as animal models in myopia research. The structural and functional of guinea pigs' retina is similar in many terms to that of humans.[21] Their photopic electroretinograms (ERGs) and critical flicker frequencies (CFF) are more similar to those of humans than those of most other widely used animal models.[22] The architecture of the choroid membrane in guinea pigs is also similar to that of human.[23] These similarities between guinea pig and human eyes make guinea pig an appropriate species for myopia research. As we known, axial length is highly correlated with spherical refractive error including myopia.[24] We found that the axial lengths of guinea pigs' eyes in L group and J light group increased rapidly compared with natural light group, while no statistically significant difference was found between E light group, B light group and natural light group. Considering light spectrum composition, low color temperature LED light was discontinuous and has 430-460 nm wavelength blue light peak as well as 480nm wavelength valley, whereas E light has continuous wavelengths ranging from approximately 390 to 780 nm with no big peak or valley. When comparing E light with J light, the spectrum profile was similar and the only difference was a small peak at 430 nm followed by a small valley, which suggested that the small peak and valley play a crucial role in eye growth. These findings can be explained by strong light suppression phenomenon. The high peak light of L group and J light group will suppress the relatively low valley light, making the latter one cannot be perceived, and forming an incoherent multifocal plane. The persistent existence of this phenomenon will produce a defocus signal different from natural light, promote the overgrowth of the eye axis, and drift to the direction of myopia. Though B light was wavelength of 400 nm below filtered, its spectrum was continuous with no peak or valley, and the eye axial growth showed no statistically significant difference comparing with natural light group and E light group. Therefore, E light was the most successful natural light imitated light with the best protective effect against eye axial elongation.

Retinal dopaminergic system has been studied extensively for its potential role in myopia inhibition. As a chemical signal of retinal photo adaptation, retinal DA release and turnover have been shown to be regulated by light in several vertebrates.[25] It is reported that detection of static retinal DA and retinal DOPAC levels cannot be representative measures of the gain of dopaminergic signaling because of the dynamic balance between DA production and metabolism. The retinal DOPAC/DA ratio, an indicator of retinal DA turnover, can represent the metabolic efficiency of DA accurately.[26] A decreased retinal DOPAC/DA ratio has been reported to be associated with an increased susceptibility to form-deprivation myopia in mice, with no changes in other DA-related levels.[27] In the present study, we found that the comparison of retinal DOPAC/DA ratios showed similar trend with that of axial lengths, indicating that retinal DOPAC/DA ratio played a crucial role in ocular growth, which was consistent with previous studies.

The question remains as to why different light spectral composition stimulate large changes in retinal DA turnover. In our study, the main distinction between the light of different groups was the component of 480 nm wavelengths lighting. Recently, the third class of photoreceptor cells of the retina in addition to rods and cones, characterized by the expression of a photoreceptive protein known as melanopsin, intrinsically photosensitive retinal ganglion cells (ipRGCs) have drawn our attention.[28,29] Interestingly, melanopsin which exert crucial effects in nonimaging visual-forming system, circadian rhythm, and activation of the pupillary light

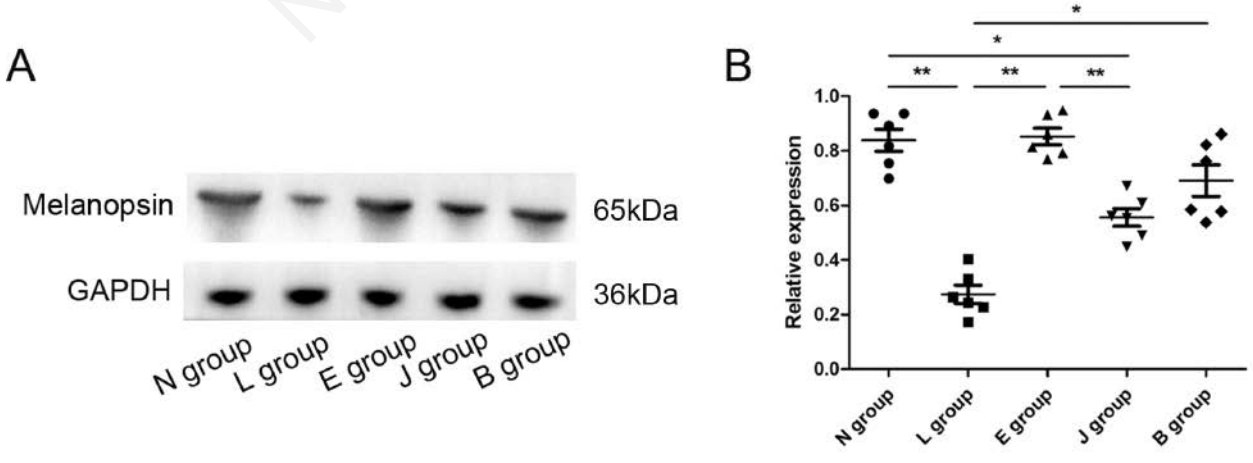

Figure 5. A) Expression of retinal melanopsin protein by Western blot analysis. B) Relative expressions of melanopsin protein of each group normalized to GAPDH were shown; *p<0.05, **p<0.01.

reflex, has a peak absorption at 479 nm.[30] It has been reported that dopamine seems to function reciprocally to melanopsin produced by ipRGCs.[31,32] We assumed that melanopsin may be involved in the process of ocular growth in our study. Melanopsin drive diurnal cycles in retina and pineal organ but do not mediate spatial vision.[33] Accordingly, they affect retinal DA and melatonin cycles. Researchers have tried to figure out the how melanopsin affect refractive development upon different light exposure, however, the specific mechanism still remain unknown. We found that retinal melanopsin expression was different among various light exposure groups, the comparison of which was consistent with that of axial lengths, indicating that retinal melanopsin may play a role in refractive development in our study.

Light induced retinal damage has been intensively studied and reported. Photoreceptors and retinal pigment epithelial (RPE) cells have gained the most attention mainly because these cell types express photosensitizers, which are able to absorb and be affected by light. [34,35] However, scientists have realized that RGCs can be damaged partly as a secondary event associated with phototoxic-induced photoreceptor loss recently.[36] Studies also have pointed out that RGCs can be directly affected by excessive light, especially the blue region (310-450 nm) of the spectrum, which has the ability to play a direct effect on RGC mitochondria.[37] We observed prominent edema, vacuolated spaces and other degenerative changes in the inner nuclear and the ganglion cell layers of artificial light group, while no remarkable pathological change was observed in the retinal specimen of natural light group.

Our study indicated that continuous full spectrum artificial light which resembled the spectral composition of natural light with no peak or valley showed the best protective effect against myopia in guinea pigs *via* retinal dopaminergic and melanopsin system. Further experiments were needed to reveal how retinal dopaminergic and melanopsin system were involved in refractive development and its specific mechanism.

Acknowledgments

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