• Basic Research •

Different color lights on refractive status and secretion of neurotransmitters in the guinea pig model of myopia

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Abstract

● **AIM:** To evaluate the effect of different monochromatic lights on the refractive status and the secretion levels of neurotransmitters in the progressive myopic model of guinea pigs.

● **METHODS:** Guinea pigs (*n*=90) underwent different monochromatic lights irradiation for two weeks were randomly divided into 6 groups: white light (control), ultraviolet (UV), blue, green, red, and simulative sunlight (simSUN). The refractive status and axial length (AL) were measured. Transmission electron microscopy, Masson's trichrome staining and hematoxylin-eosin staining were performed to observe the structural changes of retina and sclera. High-performance liquid chromatography (HPLC), western blotting and immunofluorescence were used to measure neurotransmitters and their receptors.

● **RESULTS:** Myopia models were established successfully. When compared the degrees of change in myopic eyes of control group, the UV group showed a minor decrease in AL and refraction, along with a significant increase in scleral thickness. In contrast, the red and green groups revealed a net increase in AL and refraction, coupled with a net decrease in scleral thickness (all, *P*<0.01). The dopamine concentration increased in the UV group, while concentrations of serotonin and melatonin significantly decreased (all, *P*<0.01). The groups that were exposed to UV, blue and simSUN, the expression of dopamine receptor D2 (DRD2) increased, and the expression of hydroxytryptamine receptor 2A (HTR2A) and melatonin receptor type 2 (MT2) decreased significantly when compared to the control group (all *P*<0.01).

● **CONCLUSION:** Exposure to short-wavelength light could slow the development of myopia by promoting the production of dopamine and suppressing the serotonin and melatonin concentration. The neurotransmitter receptors MT2, DRD2, and HTR2A in the sclera appear to play different roles by different color lights in myopic guinea pigs.

● **KEYWORDS:** myopia; ultraviolet light; refractive status; melatonin; melatonin receptor type 2 receptor

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INTRODUCTION

Myopia occurs when the ocular axial length (AL) is too long for the refractive power of the lens and cornea, which in turn causes the image of distant objects to be focused in front of the photoreceptor layer of the retina. Myopia can seriously affect health and quality of life. But there is no effective method to cure myopia. The etiology and pathogenesis of myopia are not completely clear. Prevention and treatment have become key research priorities in recent years. Lens-induced myopia (LIM) is a major method used to generate a guinea pig model of myopia[1-2]. Previous research found that LIM plays an important role in the regulation of signal molecules in the retina, including neurotransmitters and their receptors, and growth of the eyeball can be regulated through interactions of these signal molecules[2-3]. It has been previously demonstrated that neurotransmitters in the retina play a part in postnatal ocular development and eye growth[4-5]. Dopamine (DA) is a retinal neurotransmitter involved in the development of myopia. Retinal DA is released exclusively from amacrine or interplexiform cells, and its secretion level increases during the daytime, but declines in darkness[6-7]. Studies have shown that reduced retinal DA level occurs in experimental myopia, and this work shows that DA agonists can stimulate D2-receptor and interact with early growth response 1 to trigger the first steps in eye growth and suppress the development of LIM[8]. As a monoamine neurotransmitter,

serotonin plays multiple roles in the eyes, such as increasing intraocular pressure and causing contraction of intraocular vessels[9]. Previous studies indicate that modulation of the serotonin system influences ocular growth[10-11]. For instance, serotonin binding to its receptor can cause progressive myopia in guinea pigs[11-12].

Melatonin is a chronobiological indoleamine neurotransmitter with a marked circadian rhythm that reaches its peak in darkness and hits bottom in daylight[13-14]. Due to its circadian rhythm, melatonin plays the role of a photo-neuroendocrine transducer, regulating postnatal ocular growth, axial elongation, and emmetropization in the eye[14-15]. Melatonin mediates diverse biological rhythms in the eye through its receptors, which currently comprise three identified subtypes: melatonin receptor type 1 (MT1), MT2, and MT3[14,16]. Notably, myopic individuals exhibit higher serum melatonin concentrations than non-myopes[17]. Previous work has also demonstrated that expression of melatonin is inhibited under blue light in the eyes of guinea pigs[18].

Acetylcholine (ACh), an organic chemical that functions in the brain and body of many animals' species including humans, has five characterized subtypes of muscarinic acetylcholine receptors (mAChRs, M1-M5). Among these three subtypes (M2, M3, and M4) have been identified in the chick retina, retinal pigment epithelium, and choroid[19]. Atropine, a muscarinic ACh receptor antagonist, has been used as an anti-myopia drug, and ACh has been implicated in this signal cascade[20]. In the present study, we compare the concentration of these four neurotransmitters in the myopic model, as well as their corresponding receptors.

Natural light is polychromatic, composed of different bands of light. The wavelength of natural light ranges from 200 to 700 nm, including visible and invisible light. Torii *et al*[21] have demonstrated that violet light and ultraviolet (UV) light (360-400 nm wavelength) suppress the elongation of the AL in the chick myopia model, and the expression of myopia-suppressive gene early growth response 1 is enhanced due to UV exposure. Blue wavelength significantly decreased levels of melatonin and its receptor in the eye of tropical damselfish and guinea pigs[18,22]. Previous research has implied that myopia, especially pathological myopia, is a collagenous disease that involves remodeling of the sclera[23]. It has been reported that the occurrence of myopia is consistently accompanied by decreased amounts of scleral collagen content, as well as changes in the diameter and distribution of collagen fibers in the eye[23-24]. These studies imply that exposure to different lights may modulate the secretion of intraocular neurotransmitters and the expression of corresponding neurotransmitter receptors, thereby affecting ocular growth and the changing patterns of scleral collagens.

However, the effect of exposure to different monochromatic wavelengths of light on myopia has not been well documented. The underlying mechanism for structural change of the sclera in myopic eyes remains unclear. To better understand the progression, prevention, and treatment of myopia, the present study explores the effect of different monochromatic light on refractive status, secretion level of neurotransmitters, and structural changes in the sclera.

MATERIALS AND METHODS

Ethical Approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. The study protocol was approved by the Ethics Committee of Zhejiang Provincial People's Hospital (No.2017KY001). The animal care procedures were reviewed and approved by the Laboratory Animal Department of Hangzhou Medical College in this study, where adherence to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research was observed and enforced.

Establishment of Myopia Model and Treatment A total of 90 female guinea pigs (*Cavia porcellus*, tricolor strain), aged 4wk, were fed indoors in a standardized manner. The guinea pigs wore a -10.0 diopter (D) concave lens in the right eye for 14 consecutive days in order to establish the myopic model, with the left eye of each animal serving as a control. The lens was a -10.00 D poly(methyl methacrylate) (PMMA) lens purchased from Shanghai Freshkon Contact Lenses Co., Ltd., China, designed with a diameter of 11.00 mm and inner arc curvature of 9.00 mm.

Housing and Lighting All 90 guinea pigs were randomly divided into 6 groups (n=15 in each): white light (control, color temperature 5000 K), UV light (UV, peak value 370 nm), blue light (blue, peak value 480 nm), green light (green, peak value 530 nm), red light (red, peak value 610 nm), and simulative sunlight (simSUN, xenon arc lamp solar simulator, Oriel, Stanford, CT, USA)[18,25-28]. During the modeling period, each group of guinea pigs underwent irradiation from a different monochromatic light for 14d in a row. Intensities were controlled to 2.2 μmol/m^2·s by modulating the voltage of the light-emitting diode tubes. The irradiance in the cage was calibrated by an IL1700 Research Radiometer (International Light Inc., USA). Animals were placed under conditions of 12h of darkness and 12h of light irradiation (12D:12L). Specific light sources were used for the irradiation of animals in the 12h of lighting exposure.

Ocular Refraction and Axial Length Measurements were performed prior to as well as after the establishment of the myopic model. General anesthesia in the animals was

conducted by injecting ketamine hydrochloride into the thigh muscles. AL was measured with A-node ultrasonic diagnostic equipment (Meda Co., Ltd, China). To examine refractive errors, one drop of 1% tropicamide eye drops was added three times at an interval of ten minutes. Strip light detection on the horizontal and vertical meridians was detected at intervals of 0.5 D[29-30]. Retinoscopy optometry (Fizz Kang Visual Light Company, China) was used to examine the diopter after cycloplegic pre- and post-experiments.

Scleral Thickness Measurements Scleral thickness was measured using thin sections stained with hematoxylin and eosin under light microscopy. Photoreceptor orientation in the sections was monitored closely to avoid oblique sectioning. Effort was made to minimize the variability of thickness by taking into account the scleral thickness readings of 20 sections in a row, beginning from about 0.4 to 0.5 mm and the thickness part of the central area of the sclera tissue (posterior polar sclera tissue, at about 1.5 mm away from the temporal side of optic disc) was taken. Four electron micrographs (340 000× magnification) of approximately equal area were captured from each defined scleral layer. Each of these sample micrographs constituted a different collagen fiber bundle. Around 400 fibrils were sampled for each defined scleral layer. Fibers were measured using a digitizing tablet, and the smallest diameter was measured for elliptical fibers. Estimations of the number and mean maximum thickness of collagen fibril bundles through the sclera were obtained based on triplicate measurements.

Hematoxylin-Eosin Staining and Masson Trichrome Staining Animal eyeballs were removed after anaesthesia by pentobarbital sodium. Those eyeballs, fixed with 4% paraformaldehyde, were dehydrated within different gradient ethanol liquids (60%, 70%, 80%, 90%, 95%, 100%) for 1h. Then xylene was used twice, 30min for each time, to make those eyeballs to be transparent. Then tissues from those eyeballs were placed in three melting wax boxes covered by soft wax separately, with at 55℃ (soft wax I), 55℃ (soft wax II), 57℃ (soft wax Ⅲ) for 20min in a paraffin oven. Once becoming totally paraffinized, the tissues were embedded in a metal embedding box containing melted paraffin. After fully solidified, 5-μm-thickness eyeball paraffin sections with eyeball tissues were made using a microtome.

After being de-paraffinized and rehydrated, sections of the eyeballs were washed with distilled water. Sections were then stained with alum hematoxylin and rinsed in running tap water. 0.3% acid alcohol was used to differentiate the sections. After that, sections were rinsed in running tap water and in Scott's tap water substitute. Later, the sections were rinsed in tap water and were stained with eosin for 3min. Finally, the sections were dehydrated, cleared, and assessed.

For Masson's trichrome staining, sclera tissue sections were de-paraffinized and rehydrated through 100%, 95%, and 70% alcohol, and were washed in distilled water. Sections were rinsed using tap water for 10min to remove the yellow color, and were stained in Weigert's iron hematoxylin working solution for 10min. Afterwards, the tissue sections were rinsed in running warm tap water for 10min and washed in distilled water. The tissues were stained in Biebrich scarlet-acid fuchsin solution for 15min, and were washed in distilled water. Tissue sections were differentiated in phosphomolybdic-phosphotungstic acid solution for 15min, and then were transferred to aniline blue solution and stained for 10min. After that, the sections were rinsed briefly in distilled water and were differentiated in 1% acetic acid solution for 5min. Prior to washing in distilled water, the sections were quickly dehydrated using 95% ethyl alcohol, absolute ethyl alcohol, and xylene.

Transmission Electron Microscopy The whole eyeball was fixed in 2.5% glutaraldehyde for 1h, and then anterior segments of eyes were removed. A 2 mm×3 mm piece of retina tissue was cut along the equatorial direction, then fixed again in 2.5% glutaraldehyde for at least 24h. After that, the solution was replaced with 3% glutaraldehyde and 1% osmium tetroxide. Finally, retinal morphology was captured with transmission electron microscope.

Immunofluorescence Animals were sacrificed, and the sclera tissues of each eye (diameter, 6 mm) were taken from the posterior sclera and immediately immersed in 4% paraformaldehyde to be fixed for 24h. Formalin-fixed paraffin-embedded (FFPE) sclera tissue specimens were sequentially sectioned at 4 μm each. After de-paraffinization and rehydration, FFPE sections were treated with 0.3% H_2O_2 followed by 10% normal goat serum. Antigen retrieval was conducted using ethylene diamine tetraacetic acid (pH 8.0) at 100°C for 25min. These slides were washed and then incubated overnight at 4°C with dopamine D2 receptor (DRD2) antibody (1:100), 5-hydroxytryptamine 2A receptor (HTR2A) antibody (1:100), GPR50 antibody (1:100), and muscarinic acetylcholine receptor 2 (CHRM2) antibody (1:50). Afterwards, FFPE sections were incubated at 37°C for 40min, then washed and incubated with secondary antibody at room temperature. Images were taken using a Zeiss light microscope (Axiomat, Zeiss, Oberkochen, Germany) at various magnifications.

High-Performance Liquid Chromatography Retinas were dissected carefully from eyes. The concentrations of dopamine, serotonin, melatonin, and acetylcholine were measured by high-performance liquid chromatography (HPLC). Standard solutions and reagents were used to build calibration curves of the neurotransmitters. After that, retina tissues were

homogenized by vortexing with 4.0 mL dichloromethane. Homogenates were centrifuged at 240 g at 4°C for 10min, and supernatants were removed afterwards. Residues were evaporated under a vacuum, and 0.2 mL of the mobile phase was added to the dried residues. The mobile phase was composed of 50% acetonitrile (v/v) and was pumped at the rate of 0.5 mL/min at 30°C. Samples in the mobile phase were injected into an HPLC system equipped with an Agilent Zorbax Oligo 5 μm column (80×6.2 mm *i.d.*) and fluorometric detector (Waters, NY, USA). The fluorometric detector utilizes excitation and emission at wavelengths of 285 nm and 350 nm, respectively.

Western Blotting The eyes of animals were quickly enucleated, sclera tissue was collected, and protein concentration was determined using bicinchoninic acid quantification protein assay kit (Pierce, CA, USA), following the manufacturer's instructions. The tissue homogenate was boiled at 100°C for 5min, subsequently electrophoresed on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel and transferred to a polyvinylidene fluoride membrane (Merck Millipore, Germany). Membranes were blocked by incubating for 2h in Tris-buffered saline with Tween-20 (TBST) with 5% non-fat dry milk. Blots were incubated for 2h at room temperature (RT) with the following primary antibodies: DRD2 (cat# Rs-10081R, 1:1000, Rui Qi Bio Technology), melatonin receptor 1B (MTNR1B; encoding the MT2; cat# Rs-187857R, 1:2000, Rui Qi Bio Technology), HTR2A (cat# Rs-207420R, 1:1000, Rui Qi Bio Technology), CHRM2 (cat# ab90805, 1:2000, Abcam). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. After washing with TBST, the polyvinylidene fluoride membranes were incubated with horseradish peroxidase-conjugated secondary antibody (#A0545, 1:16 000, Sigma-Aldrich, MO, USA) for 2h at RT. After washing, bands were detected using enhanced chemiluminescence reagents (Merck Millipore, Germany). Band intensities were determined using Image Quant software.

Statistical Analysis Statistical analyses were performed by SPSS26.0 software (Chicago, IL, USA). Quantitative data are presented as mean±standard deviation. Two-way analysis of variance was applied for tests of differences among the groups. Turkey's multiple comparisons test was used to conduct group comparisons. Results were considered statistically significant at a level of $P<0.05$.

RESULTS

Changes in Axial Length and Refraction The present study aimed to establish an LIM myopia model and analyze the effects of mixed white light, UV light, blue light, green light, red light, and simulated sunlight on the development of myopia during its induction in guinea pigs.

On day 0, we measured the refraction status and ocular AL of the left and right eyes of each guinea pig. The results showed that the mean refractive value of the left eye was 3.28±0.22 D (control), 3.34±0.19 D (UV), 3.34±0.13 D (blue), 3.32±0.10 D (green), 3.39±0.10 D (red), and 3.36±0.09 D (simSun), and the mean refractive value of the right eye was 3.27±0.24 D (control), 3.32±0.19 D (UV), 3.33±0.14 D (blue), 3.34±0.13 D (green), 3.40±0.14 D (red), and 3.35±0.09 D (simSun). The results showed that the mean AL of the left eye of each group was 7.39±0.16 mm (control), 7.35±0.12 mm (UV), 7.34±0.10 mm (blue), 7.34±0.10 mm (green), 7.36±0.05 mm (red), and 7.38±0.09 mm (simSun), and the mean AL of the right eye was 7.40±0.17 mm (control), 7.34±0.13 mm (UV), 7.33±0.22 mm (blue), 7.35±0.12 mm (green), 7.36±0.08 mm (red), and 7.38±0.09 mm (simSun). There was no statistical difference between the left eye and the right eye of guinea pigs in the same group. There was no significant difference in left eye contrast, right eye contrast, and left and right eye contrast between groups, neither the refraction status nor the ocular AL (all $P>0.05$). The results indicated that there was no difference in vision between groups of guinea pigs before myopia induction and different light source irradiation.

Fourteen days later, the mean refractive value of the left eye was 2.87±0.11 D (control), 3.60±0.16 D (UV), 3.46±0.11 D (blue), 1.94±0.15 D (green), 0.68±0.10 D (red), 2.86±0.15 D (simSun), and the mean refractive value of the lens-induced eye was -2.42±0.05 D (control), -2.07±0.05 D (UV), -2.24±0.21 D (blue), -2.97±0.15 D (green), -3.33±0.11 D (red), -2.45±0.14 D (simSun), respectively. The results of refractive values of the lens-induced eye were all significantly increased compared with the control eye in the same group ($P<0.01$). Comparison of refractive values on the same side of the eyeball in different groups showed that the relative ratio of UV and blue light is relative to the inhibition in refractive change during eye development, a process that is facilitated by green and red light, in both the control eye and the lens-induced eye in this study ($P<0.01$; Figure 1A).

As shown in Figure 1B, mean AL in the lens-induced eye was 8.41±0.03 mm (control), 8.07±0.06 mm (UV), 8.27±0.20 mm (blue), 8.57±0.18 mm (green), 8.68±0.21 mm (red), and 8.42±0.13 mm (simSun) 14d later, and mean AL in the control eye was 7.60±0.07 mm (control), 7.47±0.11 mm (UV), 7.50±0.11 mm (blue), 7.86±0.16 mm (green), 8.05±0.12 mm (red), and 7.62±0.14 mm (simSun). Comparing AL in the different groups in the lens-induced eye showed that green and red light promoted the increase of AL, whereas the UV light inhibited this process of eye development ($P<0.01$; Figure 1B). There was no significant difference in the simSUN group compared with the control group ($P>0.05$).

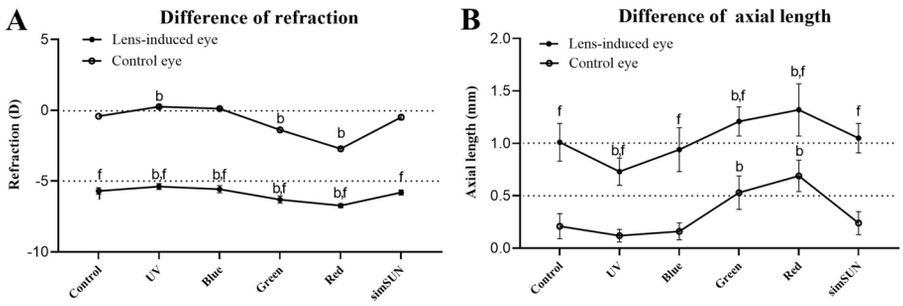

Figure 1 Myopic diopter and AL were compared between groups A: Changes in refractions between day 0 and day 14 were compared between the control eye and lens-induced eye in each group ([f]each group's control eye *P*<0.01 *vs* lens-induced eye) and compared between 6 different treatment groups in the control eye ([b]control *P*<0.01 *vs* UV, green, and red) and then in the lens-induced eye ([b]control *P*<0.01 *vs* UV, blue, green, red), *n*=15. B: Changes of AL between day 0 and day 14 were compared between the control eye and lens-induced eye in each group ([f]each group's control eye *P*<0.01 *vs* lens-induced eye) and compared between 6 different treatment groups in the control eye ([b]control *P*<0.01 *vs* green and red) and then in the lens-induced eye ([b]control *P*<0.01 *vs* UV, green, red), *n*=15. D: Diopter; UV: Ultraviolet; simSUN: Simulative sunlight; AL: Axial lengths.

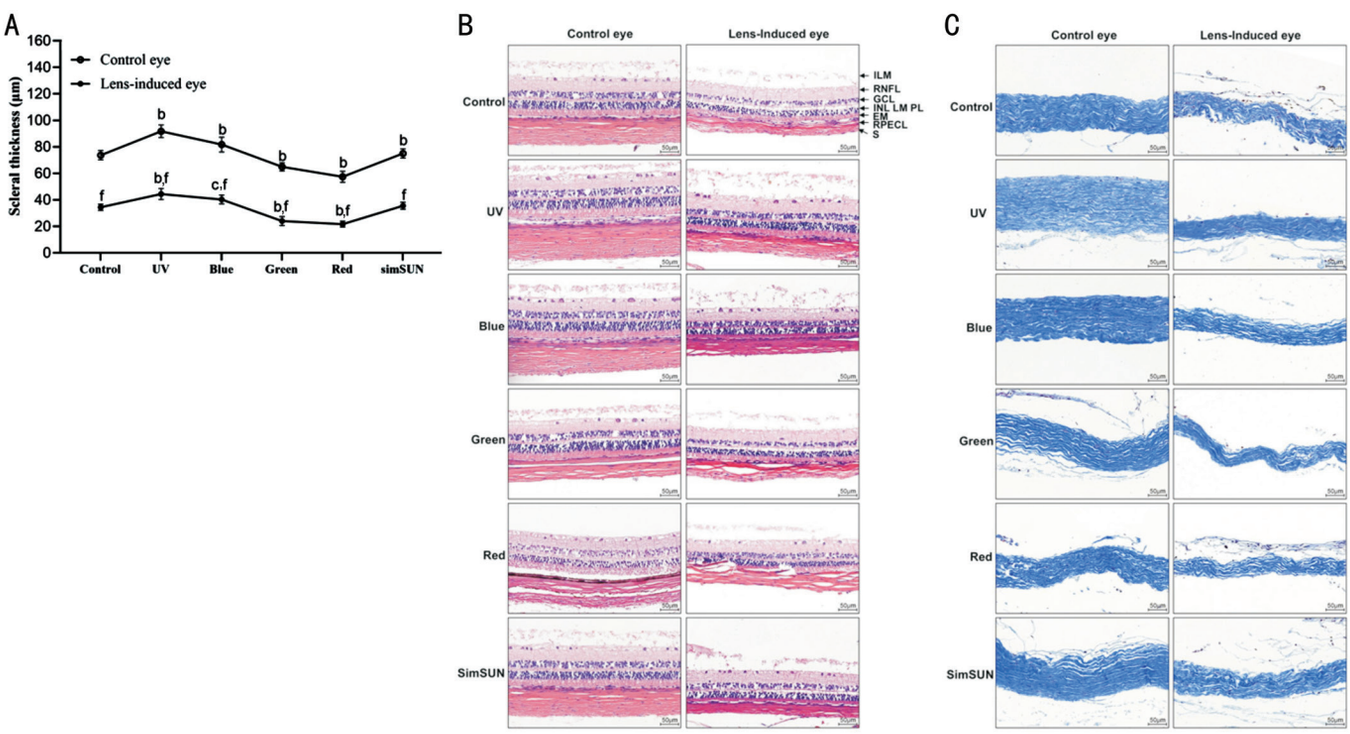

Figure 2 Measurement scleral thickness and observation sclera structure among groups A: Comparison of scleral thickness between the control eye and lens-induced eye in each group ([f]*P*<0.01 each group's control eye *vs* lens-induced eye) and compared between 6 different treatment groups in the control eye ([b]control *P*<0.01 *vs* UV, blue, green, red), and then in the lens-induced eye ([c]control *P*<0.05 *vs* blue, [b]control *P*<0.01 *vs* UV, green, red), *n*=3. B: Hematoxylin-eosin staining of retina, choroid, and sclera structure (400×), scale bar: 50 μm, *n*=3. C: Masson staining of sclera: the short-wavelength light groups exhibited a relatively normal scleral collagen fiber structure with enhanced scleral thickness and well-arranged cells compared with the long-wavelength light groups (scale bar: 50 μm), *n*=3. UV: Ultraviolet; simSUN: Simulative sunlight; ILM: Internal limiting membrane; RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; INL: Inner nuclear layer; LM: Limiting membrane; PL: Photoreceptor layer; EM: External membrane; RPECL: Retinal pigment epithelial cell layer; S: Sclera.

The results show that the induction of defocal myopia significantly increases the refraction and the AL in each group of guinea pigs. UV light could inhibit the increase of refraction and AL in the process of normal development and myopia induction. Blue light also could inhibit the increase of refraction in the process of myopia induction. Green and red light promote the increase of refraction and AL in the process of normal development and myopia induction.

Comparison of Scleral Thickness and Scleral Collagen Fiber Structure As shown in Figure 2A, mean scleral thickness in the control eye in each group were 73.71±3.51 μm (control), 91.73±4.75 μm (UV), 81.69±5.59 μm (blue),

64.88±3.09 μm (green), 57.39±4.16 μm (red), and 75.01±3.48 μm (simSun), and mean scleral thickness in the lens-induced eye was 34.44±2.38 μm (control), 44.42±4.18 μm (UV), 40.33±3.26 μm (blue), 24.07±3.38 μm (green), 21.72±2.19 μm (red), and 35.62±2.61 μm (simSun). Scleral thickness of the lens-induced eye was significantly thinner than that of the control eye in each group (P<0.01). In the control and lens-induced eye, scleral thickness was significantly thicker in the UV and blue groups and significantly thinner in the red and green groups (all P<0.01). Our data demonstrated that short-wavelength light could thicken the sclera in myopic eyes, but long-wavelength light has opposite effect. As observed in Hematoxy lin-eosin (HE) and Masson staining images in Figure 2B and 2C, the scleral collagen fiber structure of the lens-induced eye was significantly more disordered than that of the control eye in each group. In the control and lens-induced eyes, the short-wavelength light groups exhibited a relatively normal scleral collagen fiber structure with enhanced scleral thickness and well-arranged cells. However, exposure to long-wavelength light showed reduced scleral thickness and dilated intercellular space. These results suggested that scleral collagen fiber thickness and structure can be protected by short-wavelength light, but destroyed by long-wavelength light in the control and myopic eye, indicating that short-wavelength light inhibits the development of myopia, and long-wavelength light exacerbates it.

Observation of Structural Change in the Retina

Transmission electron microscopy was used to detect structural change in the retina as shown in Figure 3. Compared with the control eyes, the arrangement of rod cells in lens-induced eyes showed different degrees of disorder, as well as reduced length of rod cells. The retinal rod cells of the control and lens-induced eyes were arranged neatly in control, UV, blue, and simSUN groups, with uniform gaps between rod cells and longer rod cells. In comparison, retinal rod cells were disorderly in green and red groups, with increased gaps and reduced rod cell lengths. These results suggest that lens-induced eyes reduce the number, length, and structure of rod cells. Short-wavelength light helps to inhibit rod cell damage during the development of myopia, while long-wavelength light promotes this process.

Concentration of Neurotransmitters in the Retina Tissue

In the present study, concentrations of four neurotransmitters were compared: DA, serotonin, melatonin, and ACh. Melatonin and DA play opposing roles in the regulation of adaptive physiology of the retina. DA functions as a humoral signal for light, producing light-adaptive physiology. In contrast, melatonin exhibits dark-adaptive effects. It has been reported that the synthesis and release of melatonin and DA are under circadian control, with melatonin released at

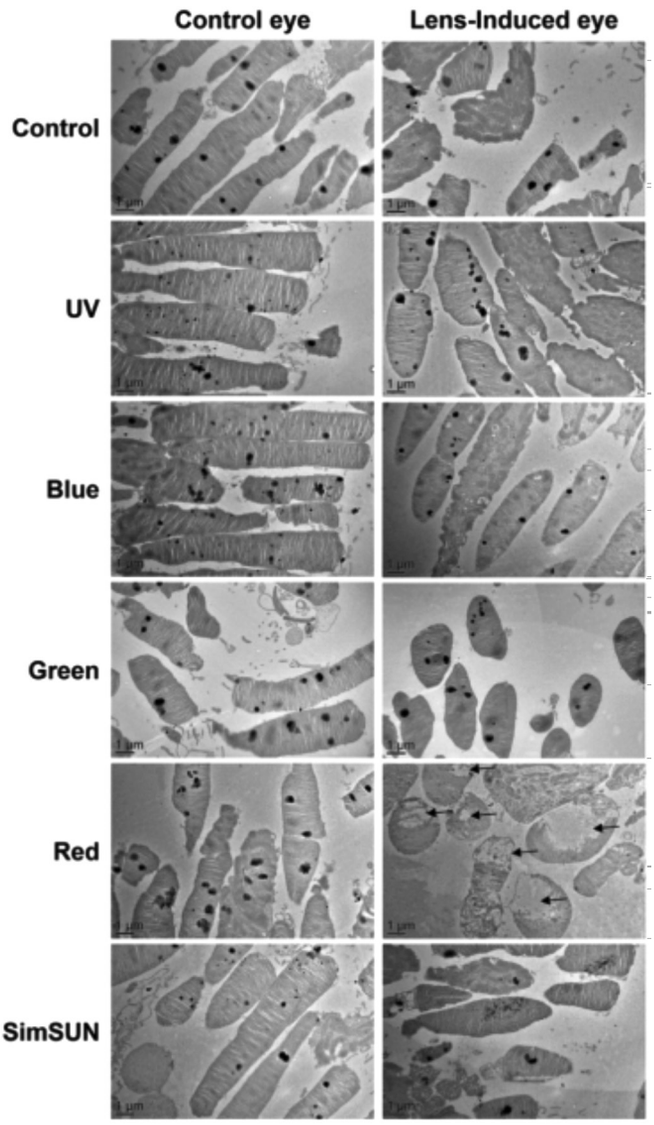

Figure 3 Observation of structural change in the retina using transmission electron microscopy (6500×) The arrangement of rod cells in different groups showed different degrees of disorder, as well as different lengths of rod cells (scale bar: 1 μm), n=3. UV: Ultraviolet; simSUN: Simulative sunlight.

night and DA during the daytime[6-7,13-14]. Figure 4 shows that every group's level of serotonin and melatonin increased significantly in the lens-induced eye, while the DA level decreased significantly (P<0.01 lens-induced eye *vs* control eye). DA in the retina significantly increased (P<0.01), while serotonin and melatonin levels significantly decreased in the UV group (P<0.01) compared with eyes in the group treated with white light. No significant difference was observed between control group and simSUN group in DA, serotonin, or melatonin levels. In the red group, DA was significantly down-regulated, and serotonin and melatonin levels were significantly up-regulated compared with the control group (P<0.01). There was no significant difference in the ACh level in the retina between any groups. These results may indicate that UV light slows the development of myopia by promoting

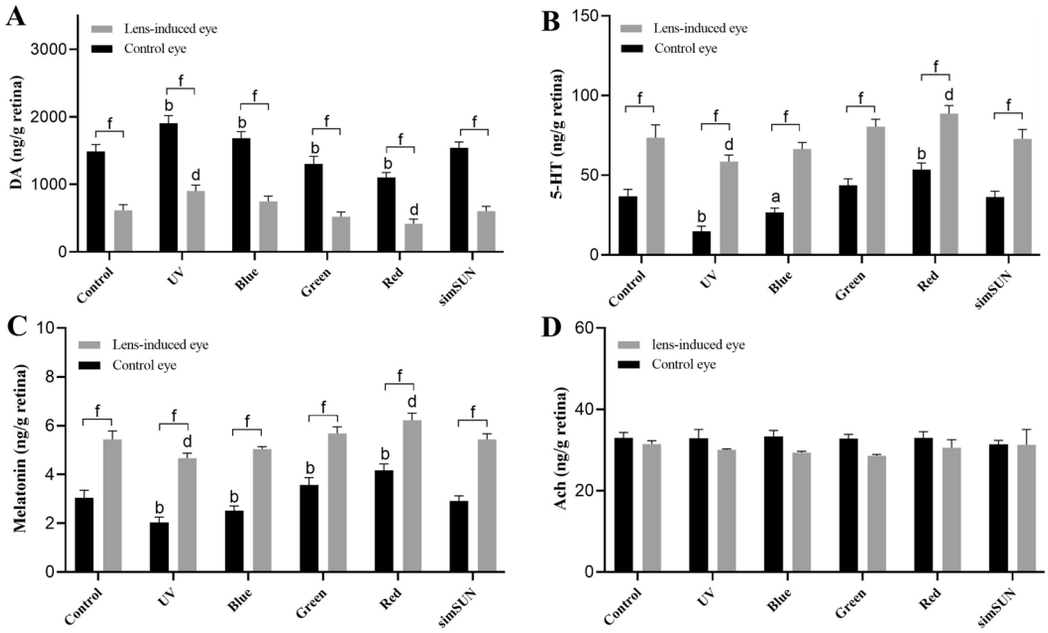

Figure 4 Comparison of neurotransmitter concentration in the retinas of guinea pigs A: Comparison of DA concentration in the retinas of guinea pigs among different groups (^{b}UV, blue, green, red P<0.01 *vs* control eye of the control group; ^{d}UV, red P<0.01 *vs* control group of lens-induced eye; ^{f}control eye P<0.01 *vs* lens-induced eye in each group), n=6. B: Comparison of the concentration of 5-HT in the retinas of guinea pigs among different groups (^{a}blue P<0.05 and ^{b}UV, red P<0.01 *vs* control eye of the control group; ^{d}UV, red P<0.01 *vs* control group of lens-induced eye; ^{f}control eye P<0.01 *vs* lens-induced eye in each group), n=6; C: Comparison of the concentration of melatonin in the retinas of guinea pigs among different groups (^{b}UV, blue, green, red P<0.01 *vs* control eye of the control group; ^{d}UV, red P<0.01 *vs* control group of lens-induced eye; ^{f}control eye P<0.01 *vs* lens-induced eye in each group), n=6. D: Comparison of the concentration of acetylcholine in the retinas of guinea pigs among different groups, n=6. UV: Ultraviolet; simSUN: Simulative sunlight; DA: Dopamine.

DA production and suppressing serotonin and melatonin concentration, but also that red light has the opposite effect.

Expression of Neurotransmitter Receptors in the Sclera

In this study, the neurotransmitter receptors DRD2, HTR2A, MT2, and CHRM2 were examined using immunofluorescence. As shown in Figure 5, DRD2 receptor expression peaked in the UV group and reached a minimum in the red group. In contrast, the expression of MT2 and HTR2A receptors showed the opposite trend. The red group showing maximum change, and the UV group showing minimum change.

As shown in Figure 6, DRD2 receptor protein expression level was significantly reduced, and HTR2A and MT2 receptors protein expression levels were significantly up-regulated in the lens-induced eye compared with the control eye (all P<0.01). No significant change was found in expression of CHRM2 receptor between the groups. Compared to the control group in the lens-induced eye, DRD2 receptor expression was significantly up-regulated, and receptors HTR2A and MT2 protein expression levels were significantly down-regulated in the UV, blue and simSUN groups (P<0.01). Meanwhile, the red and green groups produced the opposite results. These results indicated that MT2 receptors in the sclera appear to play roles in the development of myopia, and these activities can be suppressed by UV light.

DISCUSSION

In regard to structural change, scleral thinning and tissue loss at the posterior pole of the high-myopic eye were associated with abnormal ocular enlargement and myopia development[23-24]. At first, there are changes in collagen synthesis and degradation. Afterward, the diameter of the sclera collagen fibers changes, and the contents of small-diameter collagen fibers are enhanced, leading to thinner sclera. Consequently, the sclera could not withstand the imposed mechanical stresses (such as the normal intraocular pressure), the axial elongation expanded excessively, and myopia would occur[23]. When the abnormal visual stimulation from the retina reaches the sclera through the choroid, the pathological change in scleral collagens and other extracellular matrix components causes the remodeling of the sclera. Since the size and shape of the ocular are determined by the sclera, pathological change of scleral collagens is closely associated with the occurrence and progression of myopia[26]. In the current study, we found that scleral thinning and the loss of scleral tissue in the progression of myopia, as well as the alteration in the chromaticity of ambient light, can affect the progression of myopia. Moreover, irradiation with short-wavelength light decreases AL and increases scleral thickness effectively.

Riddell *et al*[31] demonstrated that illumination with

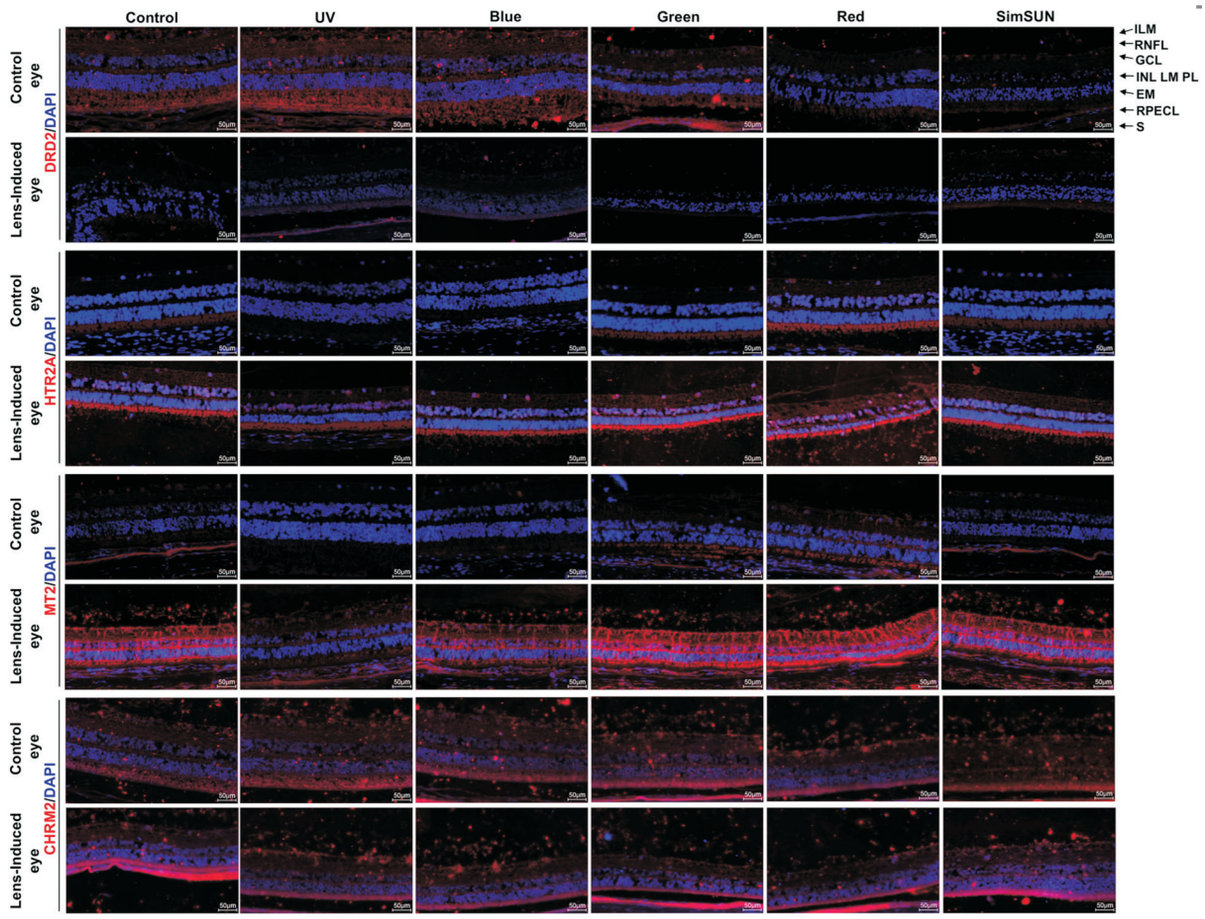

Figure 5 Expression of neurotransmitter receptors in the scler, including DRD2, HTR2A, MT2, CHRM2 (400×) Scale bar: 50 μm, *n*=3. UV: Ultraviolet; simSUN: Simulative sunlight; DRD2: Dopamine receptor D2; HTR2A: Hydroxytryptamine receptor 2A; MT2: Metatonin receptor type 2; CHRM2: Muscarinic acetylcholine receptor M2; DAPI: 4′,6-diamidino-2-phenylindole; ILM: Internal limiting membrane; RNFL: Retinal nerve fiber layer; GCL: Ganglion cell layer; INL: Inner nuclear layer; LM: Limiting membrane; PL: Photoreceptor layer; EM: External membrane; RPECL: Retinal pigment epithelial cell layer; S: Sclera.

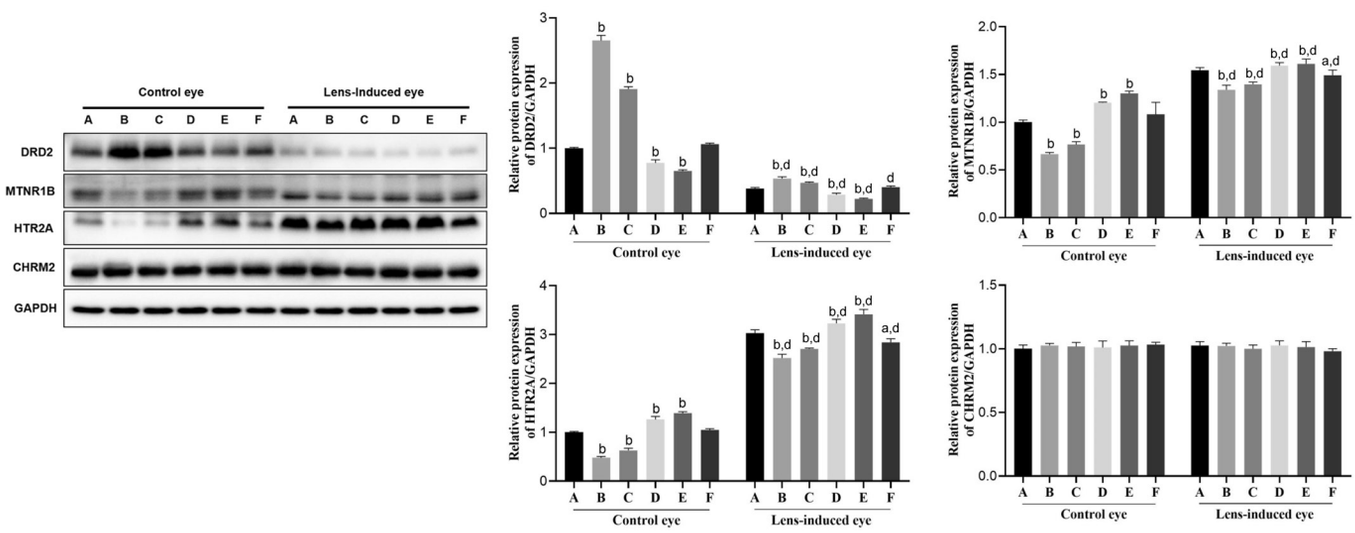

Figure 6 Western blotting analysis of DRD2, MTNR1B, HTR2A, and CHRM2 protein levels in the sclera Western blotting image (*n*=3) and quantitative comparison of protein expressions (DRD2, MTNR1B, HTR2A: [b]UV, blue, green, red *P*<0.01 *vs* control eye of the control group; [d]UV, blue, green, red, simSun *P*<0.01 *vs* control group of lens-induced eye; MTNR1B, HTR2A: [a]simSun *P*<0.05 *vs* control eye of the control group), *n*=3. A: control; B: UV; C: blue; D: green; E: red; F: simSUN. UV: Ultraviolet; simSUN: Simulative sunlight; DRD2: Dopamine receptor D2; HTR2A: Hydroxytryptamine receptor 2A; MTNR1B: Melatonin receptor type 1B; CHRM2: Muscarinic acetylcholine receptor M2; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

monochromatic long-wavelength light can give rise to myopia and cause an abnormal visual experience in chicks. The protective effect of short-wavelength light on myopia, including blue light, is supported by animal studies with molecular mechanism findings[22]. Consistent with previous research, we observed changes in AL due to exposure to different wavelengths of light. In our study, eyes exposed to long-wavelength light were rendered more highly myopic than those exposed to short-wavelength light. This model bears out the hypothesis that monochromatic short-wavelength light may alleviate or even reverse the myopic shift. Neurotransmitters in the retina play a part in postnatal ocular development and eye growth. Therefore, the present study aims to identify and compare the effects of different wavelengths of light on the secretion of neurotransmitters, the expression of neurotransmitter receptors, and the progression of myopia in the guinea pig model.

It has been previously reported that after applying negative lenses to the eyes of chickens, DA concentration decreased, while AL increased. DA regulates the growth and development of the axial portion of the eye, resulting in ceased growth when emmetropization is reached[4]. Moreover, reduced growth in the axial dimension after administration of agents interacting with DA receptors in form-deprived eyes demonstrates that retinal DA participates in the pathway linking visual experience and eye growth[32-33]. The protective effect of outdoor activity on myopia progression is partly mediated by the stimulatory effect of light on retinal DA production and release, and it was reported that DA-dependent physiological activities lead to an enhanced signal flow through the cone circuits[8]. Our results show that concentrations of DA and expression of its receptor, DRD2, are significantly higher in groups irradiated by short-wavelength light (UV or blue light) and simSUN light, compared with those groups irradiated by long-wavelength light (red or green light).

Meanwhile, significantly decreased concentrations of serotonin and its receptor HTR2A, as well as of melatonin and its receptor MT2, were found in groups treated with short-wavelength light. These observations indicate that, compared to red light, UV light enhances the secretion of DA while reducing the secretion of serotonin and melatonin in myopic eyes. Taken together, we conclude that the expression of DRD2 is inversely associated with the progression of myopia, while the expression levels of MT2 and HTR2A are positively associated with the development of myopia. These data concur with the results from the eyes of tropical damselfish observed by Takeuchi *et al*[22]. They identified the effect of light on the secretion of ocular melatonin and indicated the effective range of visible light, finding that the inhibition of ocular melatonin suppression is distributed within the wavelengths of blue light. Additionally, Wang *et al*[18] demonstrated that green light (530 nm) is involved in the development of myopia, and exposure to green light induces the production of melatonin. Therefore, MT1 receptor and melanopsin play roles in the development of myopia induced by green light in guinea pigs. Furthermore, our research analyzed the role of melatonin and a new receptor, MT2, in the progression of myopia more extensively. The exposure to short-wavelength light, such as UV light, inhibits melatonin and MT2 levels in the sclera.

Today, people spend much more time indoors than in the past, and their exposure to sunlight has decreased considerably[34]. Considerable evidence suggests that prolonged duration of outdoor activities, which increase irradiation with natural light, can effectively prevent the occurrence of myopia and inhibit its progression[35]. Natural light is composed of different bands of light, including visible and invisible light. However, the specific wavelengths of light in natural light that contribute to myopia prevention and control, as well as the related mechanisms, remain unclear. Therefore, this study comprehensively investigated the effects of different monochromatic lights within natural light exposure on myopia, as well as the related neurotransmitters. We found that short-wavelength light (especially UV light) can slow the development of myopia by reducing the AL and refraction, increasing the scleral thickness, promoting the production of dopamine and suppressing the serotonin and melatonin concentration. Zhang *et al*[36] found that ubiquitous light-emitting diode tubes may induce myopia by disrupting retinal circadian rhythms. It is speculated that the occurrence of myopia might be associated with deprivation of UV light and increased use of artificial light sources[36-38]. Given that there are no UV frequencies in the wavelengths of incandescent light, and very few UVA frequencies in fluorescent light, we propose that the potential mechanism underlying the protective effect of natural light against myopia may be attributed to the beneficial role of UV light. This finding may lay the foundation for future research into the related mechanisms and the clinical development of standardized UV lamps for daily indoor lighting to prevent myopia.

In this study, we present the changes in refractive status, secretion of neurotransmitters, and structural change of retina and sclera in myopic guinea pig models by various monochromatic wavelengths of light (Figure 7). Eyes exposed to long-wavelength light were more highly myopic than those exposed to short-wavelength light. UV light of 370 nm has been shown to be the most effective wavelength to inhibit the progression of myopia in the present study, but red light has the opposite effect. The neurotransmitter receptors MT2, DRD2 and HTR2A in the sclera appear to play different roles relative to different colors of lights in myopic guinea

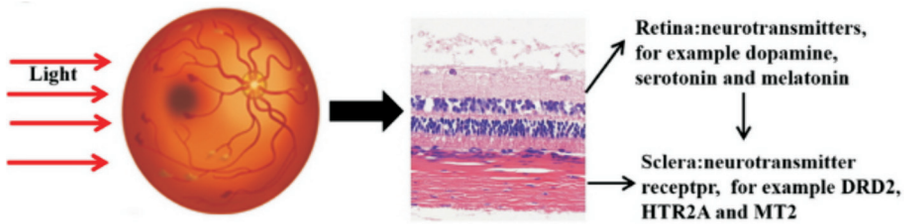

Figure 7 A schematic representation of this study DRD2: Dopamine receptor D2; HTR2A: Hydroxytryptamine receptor 2A; MT2: Melatonin receptor type 2.

pigs. Based on the results of the study, we propose that short-wavelength light affects expression of neurotransmitters and receptors, thereby inducing structural change of the sclera and AL to suppress myopia. However, further studies are needed to explore underlying mechanisms and to develop protective solutions from other effects of UV light.

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