• Basic Research •

# Effects of the long wavelength-filtered continuous spectrum on natural refractive development in juvenile guinea pigs

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## Abstract

**• AIM: To investigate the effects of spectral composition and light intensity on natural refractive development in guinea pigs.**

**• METHODS: A total of 124 pigmented guinea pigs (2-week-old) were randomly assigned to three groups at high (Hi; 4000 lx), medium (Me; 400 lx) and low (Lo; 50 lx) light intensities under a 12:12 light/dark cycle for 6wk. Each group was subdivided into subgroups with the following spectra: broad spectrum Solux halogen light (BS), 600 nm above-filtered continuous spectrum (600F), 530 nm above-filtered continuous spectrum (530F), and 480 nm above-filtered continuous spectrum (480F; HiBS: *n*=10, Hi600F: *n*=10, Hi530F: *n*=10, Hi480F: *n*=10, MeBS: *n*=10, Me600F: *n*=10, Me530F: *n*=10, Me480F: *n*=10, LoBS: *n*=11, Lo600F: *n*=12, Lo530F: *n*=10, Lo480F: *n*=11). Refractive error, corneal curvature radius, and axial dimensions were determined by cycloplegic retinoscopy, photokeratometry, and A-scan ultrasonography before and after 2, 4, and 6wk of treatment. Average changes from both eyes in the ocular parameters and refractive error were compared among different subgroups.**

**• RESULTS: After 6wk of exposure, high-intensity lighting enhanced hyperopic shift; medium- and low-intensity lighting enhanced myopic shift (*P*<0.05). Under the same spectrum, axial increase was larger in the low light intensity group than in the medium and high light intensity groups (HiBS: 0.65±0.02 mm, MeBS: 0.67±0.01 mm, LoBS: 0.82±0.02 mm; Hi600F: 0.64±0.02 mm, Me600F: 0.67±0.01 mm, Lo600F: 0.81±0.01 mm; Hi530F: 0.64±0.02 mm, Me530F: 0.67±0.01 mm, Lo530F: 0.73±0.02 mm; Hi480F: 0.64±0.01 mm, Me480F: 0.66±0.01 mm, Lo480F: 0.72±0.02 mm; *P*<0.05). Under 400 lx, there was a faster axial increase in the MeBS group than in the Me480F group (*P*<0.05). Under 50 lx, axial length changes were significantly larger in LoBS and Lo600F than in Lo530F and Lo480F (*P*<0.01).**

**• CONCLUSION: Under high-intensity lighting, high light intensity rather than spectrum distributions that inhibits axial increase. Under medium- and low-intensity lighting, filtering out the long wavelength inhibits axial growth in juvenile guinea pigs.**

**• KEYWORDS:** myopia; wavelength; spectral composition; light intensity; refractive development

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## INTRODUCTION

The prevalence rates of myopia have increased dramatically in the past decades in many regions of the world[1-5]. By 2050, approximately half of the world's population will suffer from myopia[6]. Attempts to arrest myopia progression could be dated back to centuries ago, and a variety of interventions have been tested in humans[7]. Among all interventions, outdoor exposure seems to be the most natual and economical approach. Both cross-sectional[8-10] and prospective studies[11-12] have suggested that outdoor exposure is a strong protective factor against myopia, although the exact dose-response relationship is yet to know[13-14].

French *et al*[15] assumed many factors might be linked to the protective effect demonstrated by outdoor exposure; among which one notable difference between outdoor and indoor environments is light. When comparing illumination indoors with outdoors, it's evident that sunlight provides much higher

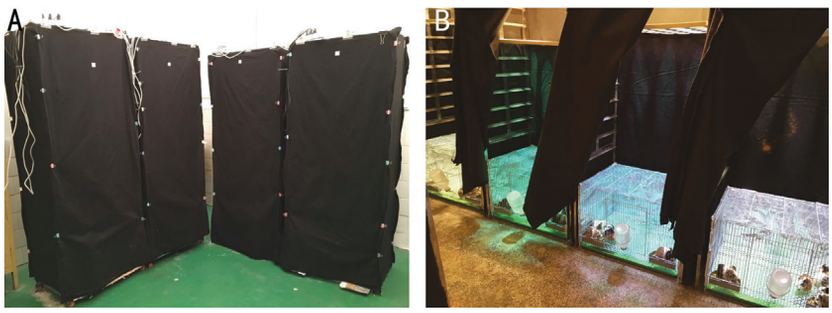


**Figure 1 Rearing cages** A: The cages covered with black shading cloth; B: Light conditions in cages.

illumination than indoor lighting even in the shade of trees or during a cloudy day[16-17]. Animal studies have also indicated that light intensity might be an important factor against myopia. The inhibition effect of high illumination was found in natural refractive development models[18] as well as in animals with lens-induced myopia[18-21] and deprivation myopia[22-23].

In addition to light intensity, sunlight differs from indoor light in spectral composition. The spectrum of sunlight includes a continuous distribution of wavelengths from approximately 300 nm to 1200 nm (adapted with permission from Kendric C Smith, ed. What is photobiology? Photobiological Sciences Online. American Society for Photobiology, http://www.photobiology.info/introduction.html.), while florescent lights, the most common source of indoor lighting, emits only a spiked distribution of wavelengths from 400 to 700 nm, with peaks in blue, green and red[18]. However, Li *et al*[18] replicated real-world lighting environments using spectrally spiked light (RGB light) and broad spectrum (BS) light and found that they had similar effects on refractive development. We speculated that although there were differences in spectral continuity between BS and RGB light sources, both of them had a broad spectral range. So it seems that the spectral composition rather than spectral continuity serves as a key factor for refractive development.

The pigmented guinea pig is one of the most commonly used mammalian models in myopia research[24-26] and has a unique wavelength-related optical system. According to some monochromatic light studies on guinea pigs, long wavelengths accelerated ocular elongation, while short wavelengths inhibited axial growth[27-31]. Therefore, we raised guinea pigs under different long wavelength-filtered continuous spectra to investigate how the differences in spectral composition and light intensity affect the refractive development.

## MATERIALS AND METHODS

**Ethical Approval** All experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the animal experimentation ethics committee of Aier School of Ophthalmology, Central South University.

**Lighting** Guinea pigs were kept in cages covered with black shading cloth (Figure 1). Solux halogen lamps (4100 K; Eiko Ltd., Shawnee, KS, USA), which emit continuous wavelengths ranging from approximately 350 to 1050 nm, were used as continuous BS lighting sources in the experiment. Since the superior retina of the guinea pig is dominated by middle wave-sensitive (M) cones (maximum absorbance, 530 nm), and all cones in the inferior retina are strongly labelled for shortwave-sensitive (S) photopigments (maximum absorbance, 400 nm)[32]. The spectral sensitivity functions curves of S cones and M cones are separated at 480 nm and do not overlap[33]. The transitional zone between these two retinal areas is populated by co-expressing cones that express both M and S cone photopigments[32]. According to the spectrum sensitivity of S and M cones, optical filters (Zeiss, Germany), which can filter out wavelengths above 600 nm, 530 nm, and 480 nm, and control filters (CR39) which allow all wavelengths to pass, were placed under the Solux light source respectively. The spectrum profile was measured with a spectrophotometer (UltraScan PRO, HunterLab, USA) by the China Branch (Zeiss, Germany). The percentages of light transmitting through the control filter substrate (Figure 2A), 600 nm short wavelength-pass filter (Figure 2B), 530 nm short wavelength-pass filter (Figure 2C) and 480 nm short wavelength-pass filter (Figure 2D) were shown in Figure 2. The intensity of illumination needed at the bottom of the rearing cages in this study was achieved by adjusting the amount and position of Solux halogen lamps. Pieces of aluminium foil were fixed around the small rearing cages to maintain homogeneous illumination. The light intensity of five positions in each cage bottom was measured with an illuminometer (T-10A, Konica Minolta, Japan) every day, ensuring that the variation of light intensity was less than 10%.

**Animals and Experimental Design** One hundred and twenty-four 2-week-old guinea pigs [Licence No. SCXK (Xiang) 2014-0010], obtained by Tian Qin Biotechnology Co., Ltd. (Hunan Province, China) were raised in a temperature-controlled room with free access to food and water. In order to investigate the effect of the light intensity and spectral property on refractive development, three levels of light intensity

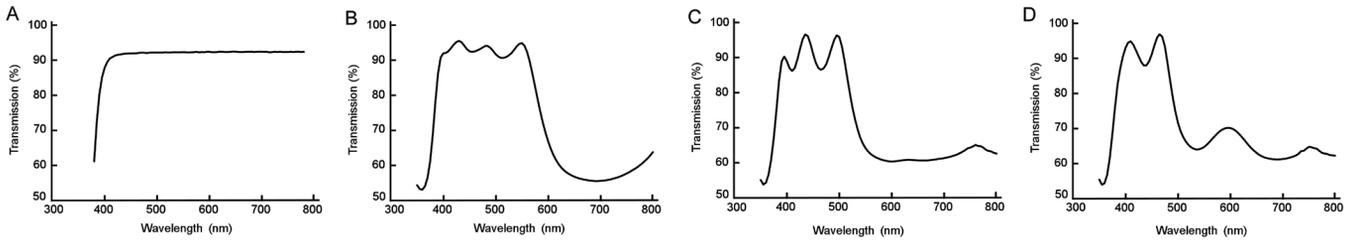


**Figure 2 Percentage of light transmitted through different filters** A: Control filter substrate; B: 600 nm short wavelength-pass filter; C: 530 nm short wavelength-pass filter; D: 480 nm short wavelength-pass filter.

combined with four different spectral composition were applied in the study. Accordingly, guinea pigs were randomly assigned to one of the following subgroups: high-intensity group (Hi; 4000 lx): 1) high-intensity with control filter substrate (HiBS; *n*=10), 2) high-intensity with 600 nm above-filtered spectrum (Hi600F; *n*=10), 3) high-intensity with 530 nm above-filtered spectrum (Hi530F; *n*=10), 4) high-intensity with 480 nm above-filtered spectrum (Hi480F; *n*=10); medium-intensity group (Me; 400 lx): 1) medium-intensity with control filter substrate (MeBS; *n*=10), 2) medium-intensity with 600 nm above-filtered spectrum (Me600F; *n*=10), 3) medium-intensity with 530 nm above-filtered spectrum (Me530F; *n*=10), 4) medium-intensity with 480 nm above-filtered spectrum (Me480F, *n*=10); low-intensity group (Lo; 50 lx): 1) low-intensity with control filter substrate (LoBS; *n*=11), 2) low-intensity with 600 nm above-filtered spectrum (Lo600F; *n*=12), 3) low-intensity with 530 nm above-filtered spectrum (Lo530F; *n*=10), 4) low-intensity with 480 nm above-filtered spectrum (Lo480F; *n*=11). The lamps for each group were switched on from 8:00 a.m. to 8:00 p.m., providing a 12-hour light/12-hour dark cycle for 6wk. The temperature was maintained between 20℃ to 24℃, and the relative humidity was controlled from 55%-65%.

**Ocular Biometry** Refractive error, corneal curvature, and axial dimensions of the eyes in each group were measured prior to the experiment and every 2wk during treatment. Refractive error: cycloplegia was induced by one drop of 0.5% proparacaine hydrochloride (Alcaine; Alcon, Fort Worth, TX, USA), followed by five drops of 0.5% tropicamide and 0.5% phenylephrine (Mydrin-P; Santen, Osaka, Japan) instilled 5min apart. The animals were held horizontally for at least 1min after each instillation to ensure that the cornea was bathed with the drug. Cycloplegic refractive error was measured using handheld streak retinoscopy (66 Vision-Tech Co., Ltd., Suzhou, Jiangsu Province, China) by two independent experienced optometrists from Aier Institute of Optometry and Vision Science who were masked with regard to the treatment. The results from the two optometrists were averaged. Refractive error was expressed as the spherical equivalent (SE), that is, spherical error plus half the cylinder error. No correction was made for the artifact of retinoscopy, which is relatively small in guinea pigs[34]. Corneal curvature: the radius of the corneal curvature was determined by a custom-made infrared photokeratometer as previously described[34-35]. Readings were accepted only when the reflection of the light emitting diode (LED) rings was centred on the pupil and all six infrared lights were seen clearly from the screen. Then, three readings were averaged to provide a value for each eye measured. Axial dimensions: the axial dimensions of the eye were measured by A-scan ultrasonography with a 10-MHz probe (KN-1800; Kangning Medical Device Co., Ltd., Wuxi, Jiangsu Province, China). One drop of 0.5% proparacaine hydrochloride (Alcaine, Alcon) was administered to the eye prior to the measurement. The ultrasound probe was placed in direct contact with the corneal apex, and special attention was paid to ensure that the probe was perpendicular to the corneal surface. The results from 10 readings were averaged for each eye measured. The vitreous chamber depth (VCD) was calculated using the following formula: VCD=axial length-anterior chamber depth-crystal lens thickness.

**Data Presentation and Analysis** All the statistical analysis was performed using SPSS 22.0 (SPSS, Chicago, IL, USA). The data were presented as mean±standard deviation (SD) unless otherwise stated. Paired *t*-tests were used to analyse the changes of ocular parameters between baseline and the end of the experiment for individual subgroups. The difference in changes among groups was compared by one-way ANOVA with the same spectral composition but different intensities or with different spectral features but the same light intensity. If significant differences were detected, post hoc tests were performed using the Bonferroni test. Pearson's correlation analysis was used to examine the relationship between the changes in refractive error and axial length. The level for statistical significance was set at two-tailed $P<0.05$.

## RESULTS

All results were based on the average data from both eyes of the guinea pigs. The average data on all ocular parameters at different time points were shown in Figure 3 and Table 1. None of the parameters, such as refractive error or axial length, were significantly different among all 12 groups at baseline ($P>0.05$).

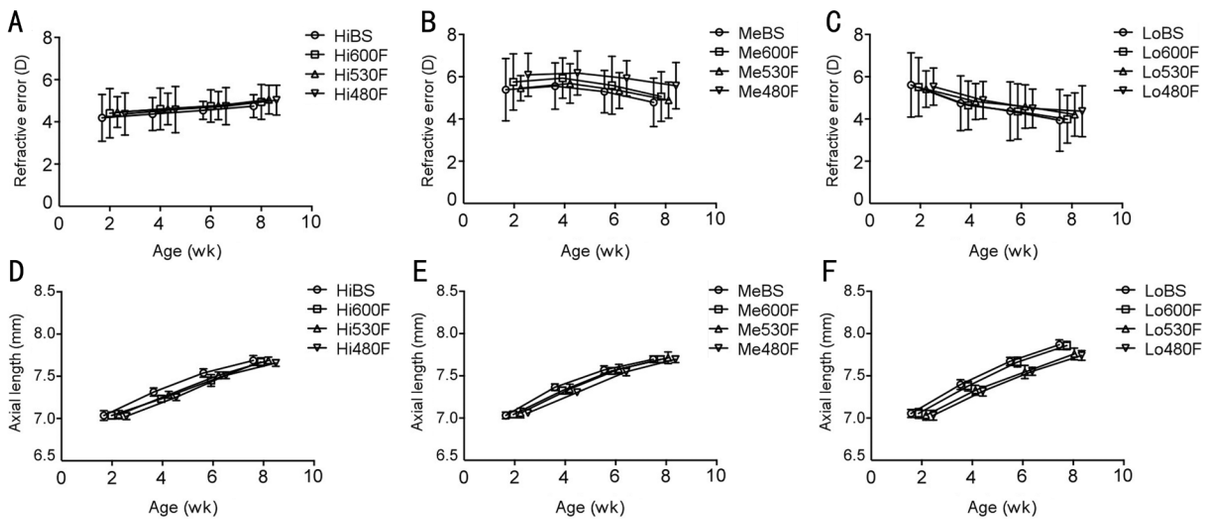


**Figure 3 Average refractive error and axial length at different time points** A: Changes in refractive error under 4000 lx; B: Changes in refractive error under 400 lx; C: Changes in refractive error under 50 lx; D: Changes in axial length under 4000 lx; E: Changes in axial length under 400 lx; F: Changes in axial length under 50 lx. BS: Solux halogen light; 600F: 600 nm above-filtered spectrum; 530F: 530 nm above-filtered spectrum; 480F: 480 nm above-filtered spectrum.

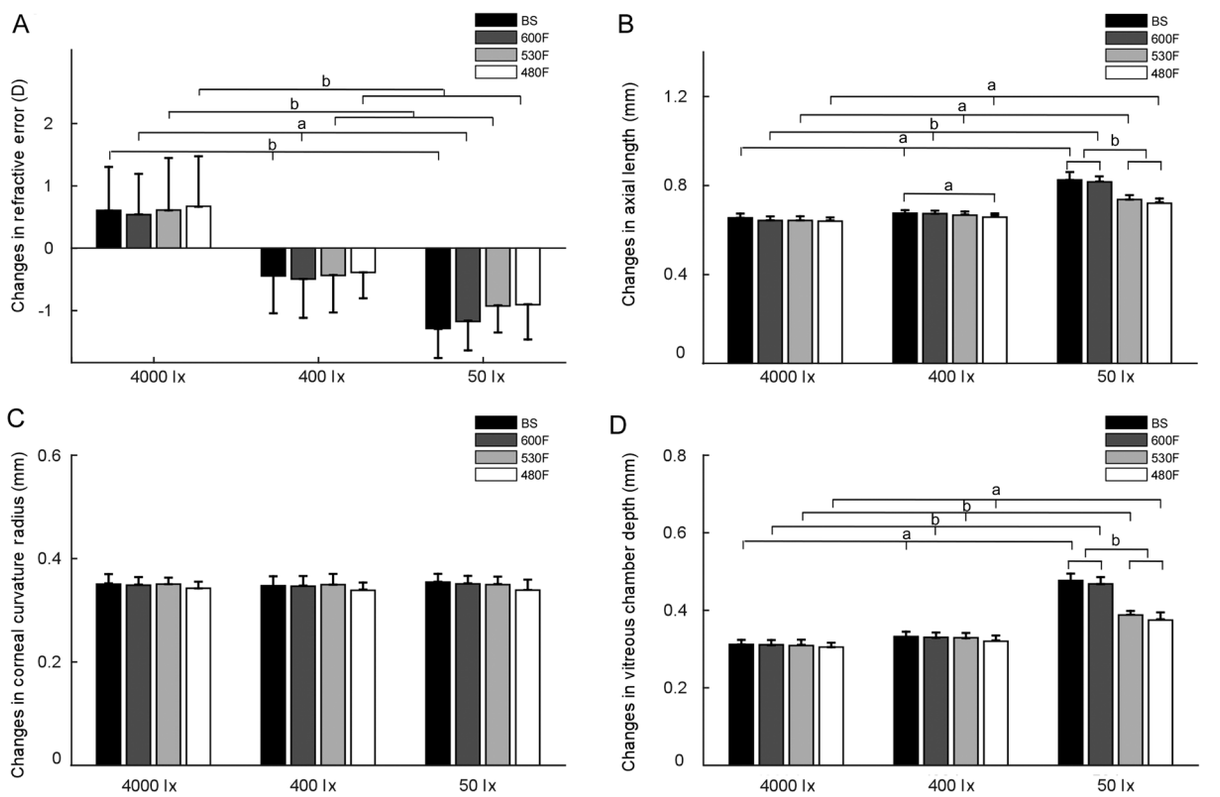


**Figure 4 Comparison of the changes in ocular parameters among the groups** A: Refractive error; B: Axial length; C: Corneal curvature radius; D: Vitreous chamber depth. BS: Solux halogen light; 600F: 600 nm above-filtered spectrum; 530F: 530 nm above-filtered spectrum; 480F: 480 nm above-filtered spectrum. Data are presented as the mean±SD. [a]$P$<0.05, [b]$P$<0.01.

**Refractive Error** At the end of the experiment, there was a significant hyperopic shift in the refractive error of guinea pigs reared in high intensity (4000 lx), while a significant myopic shift was observed in medium intensity (400 lx) and low intensity (50 lx).

With the same spectrum distributions, HiBS group exhibited a significant hyperopic shift (0.60±0.69 D), while LoBS group exhibited a significant myopic shift (-1.27±0.46 D), followed by MeBS (-0.44±0.60 D; one-way ANOVA: $F$=26.67, $P$<0.01). Similar findings were also observed for 600F (one-way ANOVA: $F$=23.99, $P$<0.01), 530F (one-way ANOVA: $F$=14.81, $P$<0.01) and 480F (one-way ANOVA: $F$=17.68, $P$<0.01) in different light intensities (Figure 4A). Post hoc tests showed that the refractive error shift differed in each

**Table 1 Changes of ocular parameters with time** mean±SD

| Groups | Subgroups | Time points | Refractive error, D | Corneal radius, mm | ACD, mm | LT, mm | VCD, mm | AL, mm |
|---|---|---|---|---|---|---|---|---|
| High intensity (4000 lx) | HiBS | Baseline | 4.19±1.11 | 3.20±0.02 | 1.17±0.01 | 2.95±0.02 | 2.93±0.04 | 7.05±0.06 |
| | | Week 6th | 4.74±0.54 | 3.55±0.01 | 1.19±0.01 | 3.27±0.03 | 3.24±0.04 | 7.71±0.06 |
| | | Change | 0.60±0.69 | 0.35±0.02 | 0.02±0.01 | 0.32±0.01 | 0.31±0.01 | 0.65±0.02 |
| | Hi600F | Baseline | 4.41±1.17 | 3.21±0.02 | 1.17±0.01 | 2.95±0.02 | 2.93±0.03 | 7.05±0.04 |
| | | Week 6th | 4.94±0.83 | 3.55±0.02 | 1.19±0.01 | 3.27±0.02 | 3.23±0.03 | 7.69±0.05 |
| | | Change | 0.53±0.64 | 0.35±0.01 | 0.02±0.01 | 0.32±0.01 | 0.31±0.01 | 0.64±0.02 |
| | Hi530F | Baseline | 4.46±0.73 | 3.21±0.03 | 1.18±0.01 | 2.96±0.02 | 2.93±0.05 | 7.06±0.05 |
| | | Week 6th | 5.06±0.68 | 3.55±0.03 | 1.19±0.01 | 3.28±0.03 | 3.24±0.04 | 7.71±0.04 |
| | | Change | 0.60±0.83 | 0.35±0.01 | 0.02±0.01 | 0.32±0.01 | 0.31±0.01 | 0.64±0.02 |
| | Hi480F | Baseline | 4.37±0.99 | 3.20±0.03 | 1.17±0.01 | 2.94±0.02 | 2.92±0.03 | 7.04±0.04 |
| | | Week 6th | 5.03±0.71 | 3.53±0.02 | 1.19±0.01 | 3.26±0.02 | 3.23±0.03 | 7.68±0.04 |
| | | Change | 0.66±0.80 | 0.34±0.01 | 0.02±0.01 | 0.32±0.01 | 0.30±0.01 | 0.64±0.01 |
| Medium intensity (400 lx) | MeBS | Baseline | 4.20±1.13 | 3.20±0.03 | 1.17±0.01 | 2.95±0.02 | 2.94±0.03 | 7.06±0.04 |
| | | Week 6th | 3.74±0.88 | 3.54±0.02 | 1.20±0.01 | 3.28±0.02 | 3.27±0.03 | 7.74±0.04 |
| | | Change | -0.44±0.60 | 0.34±0.02 | 0.02±0.01 | 0.32±0.01 | 0.33±0.01 | 0.67±0.01 |
| | Me600F | Baseline | 4.48±1.02 | 3.21±0.03 | 1.17±0.01 | 2.96±0.02 | 2.94±0.03 | 7.07±0.04 |
| | | Week 6th | 3.95±0.90 | 3.56±0.02 | 1.20±0.01 | 3.28±0.02 | 3.27±0.02 | 7.74±0.04 |
| | | Change | -0.49±0.62 | 0.34±0.02 | 0.02±0.01 | 0.32±0.01 | 0.33±0.01 | 0.67±0.01 |
| | Me530F | Baseline | 4.26±0.46 | 3.20±0.03 | 1.18±0.01 | 2.97±0.03 | 2.94±0.04 | 7.09±0.06 |
| | | Week 6th | 3.82±0.66 | 3.54±0.02 | 1.20±0.02 | 3.29±0.02 | 3.27±0.04 | 7.76±0.07 |
| | | Change | -0.44±0.59 | 0.34±0.02 | 0.02±0.01 | 0.32±0.01 | 0.33±0.01 | 0.67±0.01 |
| | Me480F | Baseline | 4.74±0.78 | 3.20±0.01 | 1.18±0.01 | 2.96±0.01 | 2.95±0.03 | 7.09±0.03 |
| | | Week 6th | 4.35±0.84 | 3.53±0.01 | 1.20±0.01 | 3.28±0.01 | 3.27±0.03 | 7.74±0.04 |
| | | Change | -0.39±0.41 | 0.33±0.01 | 0.02±0.01 | 0.32±0.01 | 0.32±0.01 | 0.66±0.01 |
| Low intensity (50 lx) | LoBS | Baseline | 4.34±1.16 | 3.23±0.02 | 1.18±0.01 | 2.96±0.02 | 2.94±0.03 | 7.08±0.05 |
| | | Week 6th | 3.06±1.12 | 3.57±0.03 | 1.20±0.02 | 3.30±0.03 | 3.41±0.03 | 7.91±0.06 |
| | | Change | -1.27±0.46 | 0.35±0.02 | 0.02±0.01 | 0.33±0.01 | 0.47±0.02 | 0.82±0.02 |
| | Lo600F | Baseline | 4.27±1.06 | 3.21±0.04 | 1.17±0.01 | 2.96±0.02 | 2.96±0.04 | 7.08±0.06 |
| | | Week 6th | 3.10±0.86 | 3.56±0.03 | 1.20±0.01 | 3.28±0.03 | 3.42±0.04 | 7.90±0.05 |
| | | Change | -1.17±0.47 | 0.35±0.01 | 0.02±0.01 | 0.33±0.01 | 0.46±0.02 | 0.81±0.01 |
| | Lo530F | Baseline | 4.19±0.66 | 3.21±0.03 | 1.17±0.01 | 2.95±0.02 | 2.94±0.04 | 7.06±0.06 |
| | | Week 6th | 3.27±0.78 | 3.56±0.03 | 1.20±0.01 | 3.28±0.02 | 3.32±0.04 | 7.80±0.07 |
| | | Change | -0.92±0.43 | 0.34±0.01 | 0.03±0.01 | 0.33±0.01 | 0.38±0.01 | 0.73±0.02 |
| | Lo480F | Baseline | 4.28±0.67 | 3.21±0.03 | 1.17±0.01 | 2.95±0.02 | 2.94±0.04 | 7.06±0.06 |
| | | Week 6th | 3.39±0.92 | 3.54±0.02 | 1.20±0.01 | 3.28±0.02 | 3.31±0.04 | 7.78±0.06 |
| | | Change | -0.90±0.60 | 0.34±0.02 | 0.02±0.01 | 0.32±0.01 | 0.37±0.02 | 0.72±0.02 |

ACD: Anterior chamber depth; LT: Lens thickness; VCD: Vitreous chamber depth; AL: Axial length. BS: Solux halogen light; 600F: 600 nm above-filtered spectrum; 530F: 530 nm above-filtered spectrum; 480F: 480 nm above-filtered spectrum.

light intensity group with the same spectrum distributions (Bonferroni test, $P<0.05$), except for comparisons between Lo530F and Me530F, Lo480F and Me480F (Bonferroni test, $P>0.05$).

Nevertheless, when comparing different spectrum distributions at the same intensity (Figure 4A), there was no significant difference among HiBS, Hi600F, Hi530F and Hi480F under high-intensity light (one-way ANOVA: $F=0.05$, $P=0.98$). Similar findings were also observed for the medium-intensity group (one-way ANOVA: $F=0.06$, $P=0.98$) and low-intensity group (one-way ANOVA: $F=1.60$, $P=0.20$).

**Corneal Curvature** The radius of corneal curvature significantly increased in all groups (paired *t*-test: all $P<0.01$; Figure 4C), with changes ranging from 0.33 to 0.35 mm. Comparing the changes among different intensity groups with the same spectrum revealed no significant difference (one-way ANOVA: BS: $F=0.35$, $P=0.71$; 600F: $F=0.44$, $P=0.65$; 530F: $F=0.02$, $P=0.98$; 480F: $F=0.18$, $P=0.84$). This was also the

case for comparisons of different spectrum groups at the same intensity (one-way ANOVA: high intensity: $F$=0.87, P=0.46; medium intensity: $F$=0.91, $P$=0.45; low intensity: $F$=1.86, $P$=0.15).

**Ocular Dimensions** The axial length of all groups increased throughout the experiment (Figure 4B, paired $t$-test, $P$<0.01), with changes ranging from 0.64 to 0.82 mm. Both light intensity and spectral composition had significant effects on the changes in axial length.

Comparing different intensity groups in the same spectrum demonstrated that the axial changes in LoBS were 0.82±0.02 mm, followed by MeBS (0.67±0.01 mm) and HiBS (0.65±0.02 mm; one-way ANOVA: $F$=271.67, $P$<0.01). Similar findings were also observed in 600F (one-way ANOVA: $F$=473.52, $P$<0.01), 530F (one-way ANOVA: $F$=82.27, $P$<0.01) and 480F (one-way ANOVA: $F$=92.00, $P$<0.01). Post hoc tests showed that the axial increase differed in each light intensity group with the same spectrum distributions (Bonferroni test, $P$<0.05).

Comparing different spectrum groups at the same intensity showed no significant difference between HiBS, Hi600F, Hi530F and Hi480F under high intensity (one-way ANOVA: $F$=1.64, $P$=0.20). However, significant differences were found within the medium-intensity groups (one-way ANOVA: $F$=4.03, $P$=0.01) and within the low-intensity groups (one-way ANOVA: $F$=96.13, $P$<0.01). In the medium-intensity groups, the axial growth in MeBS (0.67±0.01 mm) was significantly larger than those in Me480F (0.66±0.01 mm; Bonferroni test, $t$=0.02, $P$=0.02). In the low-intensity groups, the axial increase in LoBS (0.82±0.02 mm) and Lo600F (0.81±0.01 mm) belonged to one subset (post hoc analysis: $t$=0.01, $P$=1.00), whereas Lo530F (0.73±0.02 mm) and Lo480F (0.72±0.02 mm) belonged to another subset (post hoc analysis: $t$=0.02, $P$=0.28). The axial length changes in LoBS and Lo600F were significantly larger than those in Lo530F and Lo480F (post hoc analysis, $P$<0.01).

The changes in VCD were shown in Figure 4D. The outcomes of VCD changes among different groups were similar to axial length changes except for different spectral groups under 400 lx ($F$=1.68, $P$=0.19).

**Correlation Between Changes in Axial Length and Refractive Error** The correlation between the changes in axial length and refractive error for guinea pigs reared in the subgroups with different light intensities and different spectrum distributions were shown in Figure 5. Notably, the decrease in refractive error (*i.e.* more myopia) was significantly correlated with the elongation of axial length (Pearson correlated coefficient $r$=-0.67, $P$<0.01).

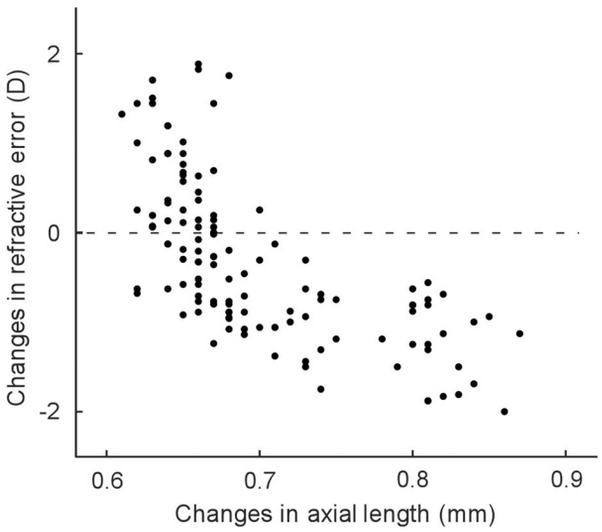


**Figure 5 The correlation between changes in axial length and refractive error.**

## DISCUSSION

In the current study, irrespective of spectrum distributions, axial length development in high light intensity was slower than that in medium and low light intensities. Additionally, high intensity induced hyperopic shifts, while medium and low intensities induced myopic shift. Within the same intensity, the effects of spectrum distributions were found in the medium- and low-intensity groups only. In the 400 lx groups, axial growth in 480F was slower than that in BS. In the 50 lx groups, the axial length changes in 530F and 480F were slower than those in BS and 600F. However, the effects of spectrum distributions were not reflected in refractive error changes.

The protective effect of high-intensity illumination found in the present study was consistent with other previous studies[18-23]. Dopamine (DA) is a neurotransmitter that inhibits the progression of myopia[19,36-38]. The synthesis and metabolism of DA in the retina are light dependent[39-40], and the inhibitory effect of high-intensity lighting on myopia can be mediated by the dopamine pathway[19]. In the current study, all subgroups exposed to 4000 lx intensity exhibited hyperopic shifts (+0.53 to +0.66 D); this result was consistent with one of our previous study[18], that the hyperopic shifts in normal refractive development of guinea pigs reared under 10 000 lx intensity ranged from +2.17 to +2.26 D, while all subgroups exposed to 400 lx intensity exhibited myopic shifts (-0.39 to -0.49 D), which was consistent with other previous researches[34,41].

The protective effects of spectral properties were only found in the 400 and 50 lx intensity groups. This may be due to the different cones which perceive both photopic and chromatic vision are oversaturated at 4000 lx intensity, and the retina cannot distinguish the excitation levels of different types of cones. At a certain high level, light intensity may play a more important role in regulating ocular growth than the spectral component. A previous study in chicks[42] also suggested that low light levels can reduce the effect of luminance cues and increase the likelihood of chromatic cues to influence the emmetropization process. Although the axial length changes

were significantly different between the BS and 480F groups under 400 lx intensity, this difference was fairly small (MeBS: 0.67±0.01 mm *vs* Me480F: 0.66±0.01 mm). In addition, the changes in VCD among groups under 400 lx were not significantly different ($P$>0.05; Figure 4D), which suggests that 400 lx may be a relatively high illuminance level for guinea pigs to utilize chromatic cues to guide refractive development. When the intensity was decreased to 50 lx, the axial changes were significantly larger in BS and 600F than in 530F and 480F. According to the spectral sensitivity curve, the M cones' function significantly decreases when the wavelength exceeds 600 nm, indicating that the guinea pig may be relatively insensitive to wavelengths above 600 nm. Therefore, the effect of the 600F group is similar to that of the BS group. Many animal studies[43-45] have proven that hyperopic defocus can promote the progression of myopia and vice versa. Therefore, the relative myopic defocus in the 530F and 480F groups due to longitudinal chromatic aberration may decrease axial growth compared with that in the BS and 600F groups. Theoretically, according to the increment-threshold spectral sensitivity functions of the guinea pig[32], the number of excited S cones and M cones should be different between the 480F and 530F groups. However, no significant difference in axial growth was found between these groups in our study. First, it may be necessary to further reduce the light intensity to highlight the effects of different spectra. Second, a monochromatic study of guinea pigs[46] indicated that co-expressing cones in the transition zone can regulate the number of M and S cones in the retina. Moreover, cone expression in the transition zone leads to plasticity in different monochromatic environments. We speculate that in the continuous spectrum under 480 nm, both S cones and co-expressing cones that express S and M cone photopigments are excited. Thus, the total S cone and M cone photopigments and corresponding opsins in the retina are similar in both the 480F and 530F groups under the regulation of co-expressing cones, which may indirectly produce similar signals regulating the eye growth.

Our study investigated the influence of different spectral compositions and light intensities in a continuous spectrum on natural refractive development in guinea pigs. However, we measured only the biological parameters of the eyeball. Moreover, the guinea pig is not a diurnal animal, and the cones are different from those in primates. Manipulations of the spectral composition have opposite effects on guinea pigs[27,29-30] compared to those on tree shrews[47-48] and rhesus monkeys[49-50]. Gawne *et al*[48] found that the infant tree shrews exposed to red light (626±10 nm) were significantly hyperopic compared with the normal ones raised in white fluorescent lighting. In another experiment[47], they found that narrow-band red light maintained this effect even in older juvenile and adolescent tree shrews. 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 normal monkeys under typical indoor lighting[49]. Hung *et al*[50] demonstrated that narrow-band long-wavelength lighting not only produced axial hyperopia, but also prevented the axial elongation produced by either form deprivation or hyperopic defocus. Therefore, the inhibitory effect of the long wavelength-filtered continuous spectrum on eye growth is typical for guinea pigs only, and extrapolation to humans may be difficult. Further molecular biological mechanism studies and experiments on primates are needed in the future.

In conclusion, under high-intensity lighting, it's high light intensity rather than spectrum distributions that inhibited axial increase. Under medium- and low-intensity lighting, filtering out the long wavelength inhibited axial growth in juvenile guinea pigs.

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**REFERENCES**

1 Pan CW, Dirani M, Cheng CY, Wong TY, Saw SM. The age-specific prevalence of myopia in Asia: a meta-analysis. *Optom Vis Sci* 2015;92(3):258-266.

2 Ma Y, Zou H, Lin S, Xu X, Zhao R, Lu L, Zhao H, Li Q, Wang L, Zhu J, He X. Cohort study with 4-year follow-up of myopia and refractive parameters in primary schoolchildren in Baoshan District, Shanghai. *Clin Exp Ophthalmol* 2018;46(8):861-872.

3 Morgan IG, Ohno-Matsui K, Saw SM. Myopia. *Lancet* 2012;379(9827):1739-1748.

4 Dolgin E. The myopia boom. *Nature* 2015;519(7543):276-278.

5 Wei S, Sun Y, Li S, Hu J, Yang X, Lin C, Cao K, Du J, Guo J, Li H, Wang N. Refractive errors in university students in central China: the Anyang university students eye study. *Invest Ophthalmol Vis Sci* 2018;59(11):4691-4700.

6 Holden BA, Fricke TR, Wilson DA, Jong M, Naidoo KS, Sankaridurg P, Wong TY, Naduvilath TJ, Resnikoff S. Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. *Ophthalmology* 2016;123(5):1036-1042.

7 Saw SM, Gazzard G, Au Eong KG, Tan DT. Myopia: attempts to arrest progression. *Br J Ophthalmol* 2002;86(11):1306-1311.

8 Rose KA, Morgan IG, Smith W, Burlutsky G, Mitchell P, Saw SM. Myopia, lifestyle, and schooling in students of Chinese ethnicity in Singapore and Sydney. *Arch Ophthalmol* 2008;126(4):527-530.

9 Dirani M, Tong L, Gazzard G, Zhang X, Chia A, Young TL, Rose KA, Mitchell P, Saw SM. Outdoor activity and myopia in Singapore teenage children. *Br J Ophthalmol* 2009;93(8):997-1000.

10 Rose KA, Morgan IG, Ip J, Kifley A, Huynh S, Smith W, Mitchell P. Outdoor activity reduces the prevalence of myopia in children. *Ophthalmology* 2008;115(8):1279-1285.

11 Saxena R, Vashist P, Tandon R, Pandey RM, Bhardawaj A, Gupta V, Menon V. Incidence and progression of myopia and associated factors in urban school children in Delhi: The North India Myopia Study (NIM Study). *PLoS One* 2017;12(12):e0189774.

12 Guo Y, Liu LJ, Tang P, Lv YY, Feng Y, Xu L, Jonas JB. Outdoor activity and myopia progression in 4-year follow-up of Chinese primary school children: The Beijing Children Eye Study. *PLoS One* 2017;12(4):e0175921.

13 He M, Xiang F, Zeng Y, Mai J, Chen Q, Zhang J, Smith W, Rose K, Morgan IG. Effect of time spent outdoors at school on the development of myopia among children in China: a randomized clinical trial. *JAMA* 2015;314(11):1142-1148.

14 Xiong S, Sankaridurg P, Naduvilath T, Zang J, Zou H, Zhu J, Lv M, He X, Xu X. Time spent in outdoor activities in relation to myopia prevention and control: a meta-analysis and systematic review. *Acta Ophthalmol* 2017;95(6):551-566.

15 French AN, Ashby RS, Morgan IG, Rose KA. Time outdoors and the prevention of myopia. *Exp Eye Res* 2013;114:58-68.

16 Wang Y, Ding H, Stell WK, Liu L, Li S, Liu H, Zhong X. Exposure to sunlight reduces the risk of myopia in rhesus monkeys. *PLoS One* 2015;10(6):e0127863.

17 Wu PC, Chen CT, Lin KK, Sun CC, Kuo CN, Huang HM, Poon YC, Yang ML, Chen CY, Huang JC, Wu PC, Yang IH, Yu HJ, Fang PC, Tsai CL, Chiou ST, Yang YH. Myopia prevention and outdoor light intensity in a school-based cluster randomized trial. *Ophthalmology* 2018;125(8):1239-1250.

18 Li W, Lan W, Yang S, Liao Y, Xu Q, Lin L, Yang Z. The effect of spectral property and intensity of light on natural refractive development and compensation to negative lenses in guinea pigs. *Invest Ophthalmol Vis Sci* 2014;55(10):6324-6332.

19 Ashby RS, Schaeffel F. The effect of bright light on lens compensation in chicks. *Invest Ophthalmol Vis Sci* 2010;51(10):5247-5253.

20 Zheng H, Tse DY, Tang X, To C, Lam TC. The interactions between bright light and competing defocus during emmetropization in chicks. *Invest Ophthalmol Vis Sci* 2018;59(7):2932-2943.

21 Smith EL 3rd, Hung LF, Arumugam B, Huang J. Negative lens-induced myopia in infant monkeys: effects of high ambient lighting. *Invest Ophthalmol Vis Sci* 2013;54(4):2959-2969.

22 Ashby R, Ohlendorf A, Schaeffel F. The effect of ambient illuminance on the development of deprivation myopia in chicks. *Invest Ophthalmol Vis Sci* 2009;50(11):5348-5354.

23 Smith EL 3rd, Hung LF, Huang J. Protective effects of high ambient lighting on the development of form-deprivation myopia in rhesus monkeys. *Invest Ophthalmol Vis Sci* 2012;53(1):421-428.

24 Chen BY, Wang CY, Chen WY, Ma JX. Altered TGF-β2 and bFGF expression in scleral desmocytes from an experimentally-induced myopia guinea pig model. *Graefes Arch Clin Exp Ophthalmol* 2013;251(4):1133-1144.

25 Zeng G, Bowrey HE, Fang J, Qi Y, McFadden SA. The development of eye shape and the origin of lower field myopia in the guinea pig eye. *Vision Res* 2013;76:77-88.

26 Zhi Z, Pan M, Xie R, Xiong S, Zhou X, Qu J. The effect of temporal and spatial stimuli on the refractive status of guinea pigs following natural emmetropization. *Invest Ophthalmol Vis Sci* 2013;54(1):890-897.

27 Liu R, Qian YF, He JC, Hu M, Zhou XT, Dai JH, Qu XM, Chu RY. Effects of different monochromatic lights on refractive development and eye growth in guinea pigs. *Exp Eye Res* 2011;92(6):447-453.

28 Seidemann A, Schaeffel F. Effects of longitudinal chromatic aberration on accommodation and emmetropization. *Vision Res* 2002;42(21):2409-2417.

29 Qian YF, Dai JH, Liu R, Chen MJ, Zhou XT, Chu RY. Effects of the chromatic defocus caused by interchange of two monochromatic lights on refraction and ocular dimension in guinea pigs. *PLoS One* 2013;8(5):e63229.

30 Jiang L, Zhang S, Schaeffel F, Xiong S, Zheng Y, Zhou X, Lu F, Qu J. Interactions of chromatic and lens-induced defocus during visual control of eye growth in guinea pigs (Cavia porcellus). *Vision Res* 2014;94:24-32.

31 Foulds WS, Barathi VA, Luu CD. Progressive myopia or hyperopia can be induced in chicks and reversed by manipulation of the chromaticity of ambient light. *Invest Ophthalmol Vis Sci* 2013;54(13):8004-8012.

32 Parry JW, Bowmaker JK. Visual pigment coexpression in Guinea pig cones: a microspectrophotometric study. *Invest Ophthalmol Vis Sci* 2002;43(5):1662-1665.

33 Jacobs GH, Deegan JF 2nd. Spectral sensitivity, photopigments, and color vision in the guinea pig (Cavia porcellus). *Behav Neurosci* 1994;108(5):993-1004.

34 Howlett MH, McFadden SA. Emmetropization and schematic eye models in developing pigmented guinea pigs. *Vision Res* 2007;47(9):1178-1190.

35 Howlett MH, McFadden SA. Form-deprivation myopia in the guinea pig (Cavia porcellus). *Vision Res* 2006;46(1-2):267-283.

36 Iuvone PM, Tigges M, Stone RA, Lambert S, Laties AM. Effects of apomorphine, a dopamine receptor agonist, on ocular refraction and axial elongation in a primate model of myopia. *Invest Ophthalmol Vis Sci* 1991;32(5):1674-1677.

37 Lin Z, Chen X, Ge J, Cui D, Wu J, Tang F, Tan J, Zhong X, Gao Q. Effects of direct intravitreal dopamine injection on sclera and retina in form-deprived myopic rabbits. *J Ocul Pharmacol Ther* 2008;24(6):543-550.

38 Mao J, Liu S, Qin W, Li F, Wu X, Tan Q. Levodopa inhibits the development of form-deprivation myopia in guinea pigs. *Optom Vis Sci* 2010;87(1):53-60.

39 Cohen Y, Peleg E, Belkin M, Polat U, Solomon AS. Ambient illuminance, retinal dopamine release and refractive development in chicks. *Exp Eye Res* 2012;103:33-40.

40 Luft WA, Iuvone PM, Stell WK. Spatial, temporal, and intensive determinants of dopamine release in the chick retina. *Vis Neurosci* 2004;21(4):627-635.

41 Zhou X, Qu J, Xie R, Wang R, Jiang L, Zhao H, Wen J, Lu F. Normal development of refractive state and ocular dimensions in guinea pigs. *Vision Res* 2006;46(18):2815-2823.

42 Rucker FJ, Wallman J. Cone signals for spectacle-lens compensation: differential responses to short and long wavelengths. *Vision Res* 2008;48(19):1980-1991.

43 Benavente-Pérez A, Nour A, Troilo D. Axial eye growth and refractive error development can be modified by exposing the peripheral retina to relative myopic or hyperopic defocus. *Invest Ophthalmol Vis Sci* 2014;55(10):6765-6773.

44 Smith EL 3rd, Kee CS, Ramamirtham R, Qiao-Grider Y, Hung LF. Peripheral vision can influence eye growth and refractive development in infant monkeys. *Invest Ophthalmol Vis Sci* 2005;46(11):3965-3972.

45 Howlett MH, McFadden SA. Spectacle lens compensation in the pigmented guinea pig. *Vision Res* 2009;49(2):219-227.

46 Hu M, Hu Z, Xue L, Yang Z, Zheng Z, He D, Zhang X. Guinea pigs reared in a monochromatic environment exhibit changes in cone density and opsin expression. *Exp Eye Res* 2011;93(6):804-809.

47 Gawne TJ, Ward AH, Norton TT. Long-wavelength (red) light produces hyperopia in juvenile and adolescent tree shrews. *Vision Res* 2017;140:55-65.

48 Gawne TJ, Siegwart JT Jr, Ward AH, Norton TT. The wavelength composition and temporal modulation of ambient lighting strongly affect refractive development in young tree shrews. *Exp Eye Res* 2017;155:75-84.

49 Smith EL 3rd, Hung LF, Arumugam B, Holden BA, Neitz M, Neitz J. Effects of long-wavelength lighting on refractive development in infant rhesus monkeys. *Invest Ophthalmol Vis Sci* 2015;56(11):6490-6500.

50 Hung LF, Arumugam B, She Z, Ostrin L, Smith EL 3rd. Narrow-band, long-wavelength lighting promotes hyperopia and retards vision-induced myopia in infant rhesus monkeys. *Exp Eye Res* 2018;176:147-160.