The Effect of Spectral Property and Intensity of Light on Natural Refractive Development and Compensation to Negative Lenses in Guinea Pigs

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Purpose. To investigate the effect of spectral composition and light intensity on refractive development in guinea pigs.

Methods. One-week-old guinea pigs were randomly assigned to groups exposed to broad-spectrum Solux halogen light (BS) or spiked-spectrum fluorescent light (FL) at both high (Hi, 10,000 lux) and low (Lo, 500 lux) intensities under a 12:12 light/dark cycle. Half of the animals in each group were used as controls (n = 24, 20, 22, and 20, respectively), and half were fitted with binocular −4-diopter (D) lenses (L, lenses; n = 22, 20, 24, and 22, respectively). Refractive error, corneal curvature, and axial dimensions were determined by cycloplegic retinoscopy, photokeratometry, and A-scan ultrasonography, respectively.

Results. Guinea pigs exposed to FL and BS showed similar changes in refraction under both high (HiFL: 2.26 ± 0.55 D versus HiBS: 2.17 ± 0.65 D, $P > 0.05$)- and low-intensity lighting (LoFL: 1.39 ± 0.88 D versus LoBS: 1.40 ± 0.93 D, $P > 0.05$). This was also true for the groups wearing lenses (HiFL-L: −1.81 ± 0.73 D versus HiBS-L: −1.45 ± 0.99 D, $P > 0.05$; LoFL-L: −2.58 ± 0.65 D versus LoBS-L: −2.29 ± 0.50 D, $P > 0.05$). Nevertheless, animals under high-intensity lighting exhibited a significantly larger hyperopic shift compared with those under low-intensity lighting (HiFL versus LoFL: $P < 0.01$; HiBS versus LoBS: $P < 0.05$). Similarly, a significantly smaller myopic shift was observed with brighter light in the lens condition (HiFL-L versus LoFL-L: $P < 0.05$; HiBS-L versus LoBS-L: $P < 0.05$).

Conclusions. In guinea pigs, spectrally spiked light and broad-spectrum light have similar effects on natural refractive development and negative lens compensation. As found in other species, effects of light intensity on refractive development were also observed in guinea pigs in both illuminants.

Keywords: guinea pigs, spectral property, light intensity, lens-induced myopia

From increasing evidence, outdoor exposure is considered to be a strong protective factor against myopia. First, a series of epidemiological studies observed that children who spent more time outdoors were less likely to become myopic.[1–4] A comparison of children of Chinese ethnicity growing up in Singapore and Sydney suggested that differences in time outdoors were the main explanation for the large differences in the prevalence of myopia in the two groups.[5] Further, it was reported that indoor sports did not provide protection against myopia,[1,5,6] indicating that physical sport is not the primary reason for the beneficial effect of outdoor exposure. In addition, myopia progression was found to be slower in the summer, when daylight hours are longer and average light intensity is higher than in the winter.[7–10] With these data taken together, it seems very likely that the quantity of time spent outdoors is associated with the risk of myopia development.[11,12]

Outdoor and indoor visual experiences are fundamentally different. Therefore, many factors might contribute to the protective effect demonstrated by outdoor exposure (see Ref. 11 for review). One of the many potential factors is the distinct difference in lighting between outdoor and indoor environments. In the first place, sunlight provides much higher illumination than most indoor lighting. In Guangzhou, for instance, illumination outdoors ranges from 13,000 to 18,000 lux in the shade to over 100,000 lux in direct sunlight at noon on a clear sunny day. In contrast, indoor illumination provided by artificial lighting is usually in the range of 300 to 600 lux. Recent findings in animals indicate that significant differences in light intensity might be an important factor contributing to the protective effect of outdoor exposure against myopia. Chickens raised under high illumination (10,000 lux) were found to develop relative hyperopia compared to those raised under medium illumination (500 lux), while chickens under low illumination (50 lux) became relatively myopic.[13] Moreover, simply increasing the ambient light intensity from 500 to 15,000 lux has been shown to significantly inhibit the development of deprivation myopia in chickens,[13–15] tree shrews (Siegwart JT,

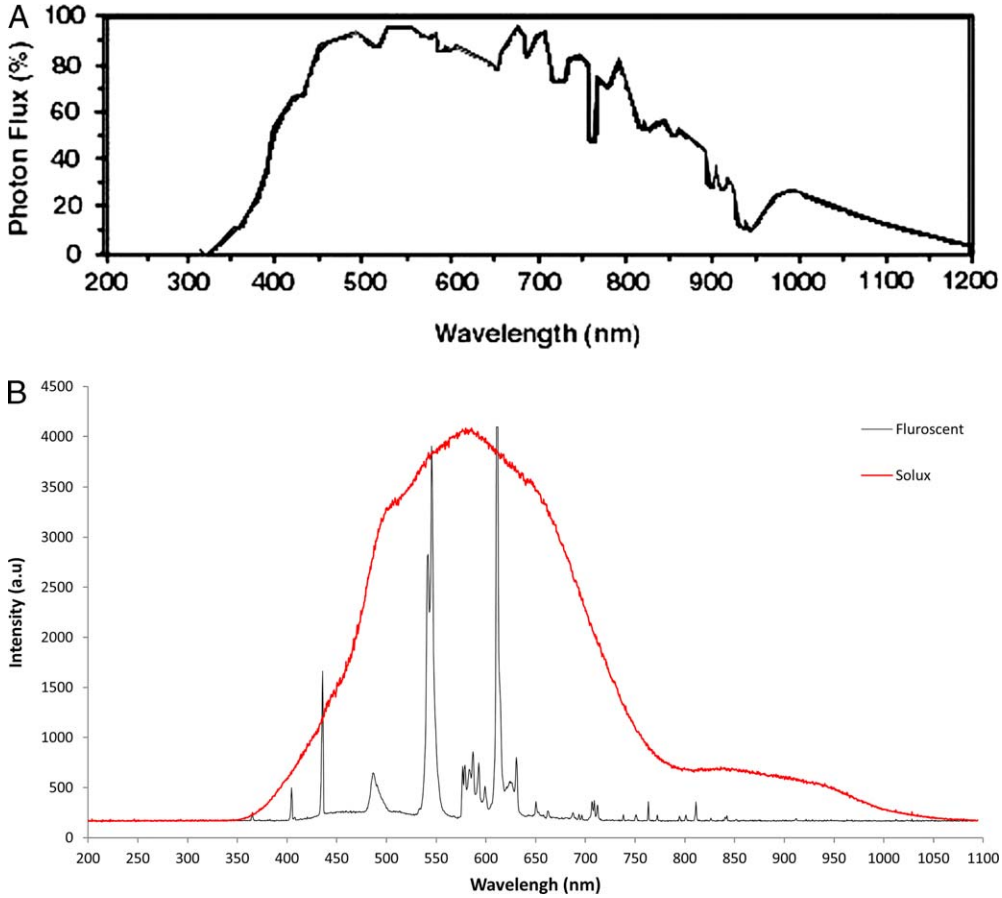

Figure 1. The spectrum of ground-level sunlight on a clear summer day (**A**) and the spectrum of Solux halogen lamp and fluorescent lamp (**B**). The spectrum of ground-level sunlight includes continuous radiation ranging from approximately 300 to 1200 nm. Adapted with permission from Smith KC, ed., *What is photobiology?* Photobiological Sciences Online. American Society for Photobiology, 2014. http://www.photobiology.info/introduction.html. Copyright August 22, 2014 Dr. Kendric C. Smith. Similarly, the Solux halogen lamp emits continuous radiation from approximately 350 to 1050 nm, which resembles the ground-level sunlight in terms of wavelength and spectrum distribution (except at radiation levels between 300 and 350 nm). By contrast, the fluorescent lamp emits only discrete rays, peaking at approximately 440, 550, and 620 nm, and the spectrum does not extend into UV and infrared regions.

et al. *IOVS* 2012;53:ARVO E-Abstract 3457), and rhesus monkeys.[16] Bright light also slowed down the development of lens-induced myopia in chickens[15] and tree shrews (Siegwart JT, et al. *IOVS* 2012;53:ARVO E-Abstract 3457), but this was not seen in rhesus monkeys.[17] In addition to light intensity, sunlight differs from indoor light in spectral composition. The spectrum of sunlight on earth during a typical day includes a continuous distribution of wavelengths from approximately 300 nm to approximately 1200 nm (Fig. 1A), as the stratospheric ozone layer filters out radiation lower than 295 nm, and radiation above 1200 nm is strongly absorbed by atmospheric water. In contrast, florescent lights, the most common source of artificial indoor lighting, emit only a spiked distribution of wavelengths from 400 to 700 nm, with peaks in the blue, green and red, and lack ultraviolet and infrared wavelengths.

As proposed previously,[16] in addition to absolute intensity, the spectral composition and distribution of light could also be critical for the protective effect from myopia observed with bright light treatment. The influence of spectral property on ocular growth has been investigated in animals by comparing the effect of different monochromatic lighting conditions.[18–25] In general, long wavelengths accelerate ocular elongation while short wavelengths inhibit ocular elongation. However, monochromatic light illumination exists only in laboratories, whereas daily illumination provided by sunlight or artificial indoor light usually consists of polychromatic spectra. In the present study, we have used two types of commercial lighting with distinct spectral properties to replicate real-world lighting environments to investigate if spectral differences are likely to have a role in the development of myopia.

Materials and Methods

Lighting

Two commercial lamps, a Solux halogen lamp (4100K; Eiko Ltd., Shawnee, KS, USA) and a fluorescent lamp (CFL23/PAR38, 4100K; Eiko Ltd.), were used as the lighting sources in the experiment. Figure 1B shows the spectrum profile of these two lamps measured with a fluorospectrophotometer (HR2000; Ocean Optics, Inc., Osaka, Japan; detection limit is 200–1100 nm) by the Department of Physics of Sun Yat-sen University in Guangzhou, China. It is noted that the Solux halogen lamp emits continuous wavelengths ranging from approximately 350 to 1050 nm (Fig. 1B). As shown in Figure 1A, the spectrum emitted by this lamp mimics the spectral composition of natural light very well except at wavelengths between 300 and 350 nm. In contrast, the fluorescent lamp emits only a discontinuous spectrum, with pronounced peaks at approximately 440, 550, and 620 nm. The spectrum does not extend into the UV and

infrared regions (Fig. 1B). To achieve the intensity of illumination needed in this study for the low (500 lux)- and high-intensity (10,000 lux) fluorescent lighting, we set three 9-W fluorescent lamps at a height of approximately 1 m above the cage and six 23-W fluorescent lamps at a height of approximately 50 cm, respectively. For the low (500 lux) and high (10,000 lux) Solux halogen lighting, we set one 50-W Solux halogen lamp at approximately 1 m above the cage and six 50-W Solux halogen lamps at a height of approximately 50 cm, respectively.

Animals and Experimental Design

The pigmented guinea pig (*Cavia porcellus*) is one of the most common mammalian models in myopia research.[23,26–33] More importantly, it has a unique wavelength-related optical system. The guinea pig has two cone types: M cones and S cones. The M-cone pigment has peak sensitivity at approximately 530 nm,[34] and the S-cone pigment has peak sensitivity at approximately 430 nm[35] (a more recent study[34] showed that the peak sensitivity for the S cone is approximately 400 nm). Thus, the S pigment is violet sensitive. Unlike what is observed in primates, the numbers of S cones in the guinea pig retina are unexpectedly high: Although the dorsal retina is dominated by M cones, having only approximately 5% S cones, all cones in the ventral retina are labeled strongly for the S pigment.[34] Furthermore, wavelengths longer than 280 nm are readily transmitted by the guinea pig cornea[36]; and although the crystal lens absorbs wavelengths shorter than 350 nm, it has a steep slope of increasing transmission for longer wavelengths including near UV (especially from 380 to 400 nm).[37,38] Consequently, the optical components of the guinea pig eye, in combination with the abundance of S pigment in the ventral retina, allows the guinea pig to have UV vision for at least wavelengths between 380 nm and 400 nm. The major difference between ground-level sunlight and the solar halogen light is the inclusion of wavelengths between 300 and 350 nm, but these wavelengths are absorbed by the crystalline lens of the guinea pig. The solar halogen light therefore reaches the guinea pig retina and stimulates the cone photoreceptors in the same way that sunlight does. The guinea pigs in the study were obtained by the Animal Experimental Centre of Zhejiang Province, China, and were raised in a temperature-controlled room with free access to food and water. In order to investigate the influence of the spectral property and light intensity on natural refractive development and refractive development affected by negative lenses, two paradigms with four different groups each were used in the experiment. Accordingly, 1-week-old guinea pigs were assigned randomly to one of the following groups.

Normal refractive development (paradigm 1): Guinea pigs were raised under one of four lighting conditions: (1) high-intensity broad-spectrum lighting (10,000 lux) of Solux halogen light (HiBS, $n = 24$); (2) high-intensity spiked-spectrum lighting (10,000 lux) of fluorescent light (HiFL, $n = 20$); (3) low-intensity broad-spectrum lighting (500 lux) of Solux light (LoBS, $n = 22$); (4) low-intensity spiked-spectrum lighting (500 lux) of fluorescent light (LoFL, $n = 20$).

Refractive development with negative lenses (paradigm 2): Guinea pigs continuously wore −4-diopter (D) lenses binocularly (L, lenses) and were raised under one of the light conditions described above: (1) high-intensity broad-spectrum lighting (10,000 lux) of Solux light with lenses (HiBS-L, $n = 22$); (2) high-intensity spiked-spectrum lighting (10,000 lux) of fluorescent light with lenses (HiFL-L, $n = 20$); (3) low-intensity broad-spectrum lighting (500 lux) of Solux light with lenses (LoBS-L, $n = 24$); (4) low-intensity spiked-spectrum lighting (500 lux) of fluorescent light with lenses (LoFL-L, $n = 22$).

The lamps for each group were switched on from 8:00 AM to 8:00 PM, giving a 12-hour light/12-hour dark cycle, for 3 weeks. The temperature was controlled to 22 ± 2°C. 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 the Zhongshan Ophthalmic Center.

Wearing of Lenses

Pieces of Velcro were modified into face masks and glued to the faces of the guinea pigs, leaving the eyes, nose, mouth, and ears exposed, as described by Howlett and McFadden.[39] Then a negative lens (−4.00 D, PMMA, diameter 18.0 mm, optical zone 12.0 mm, base curve 8.0 mm), which was already glued onto a plastic frame with Velcro, was attached to the face mask around the eye, and the optical center of the lens was aligned with the center of the pupil. The lenses were worn continuously during the experiments except when they were removed for cleaning with water-wetted gauze once a day at the commencement of the dark phase. The face masks were examined and reattached whenever necessary. In addition, whenever the lens was found to have visible scratches at its center, it was immediately replaced.

Ocular Biometry

Refractive error, corneal curvature, and axial dimensions of the eyes in each group were determined prior to the experiment and once a week for the 3 weeks of treatment.

Refractive error: Cycloplegic refractive error was measured using handheld streak retinoscopy (66 Vision-Tech Co., Ltd., Suzhou, Jiangsu Province, China) by two independent experienced optometrists from Zhongshan Ophthalmic Center, who were masked with regard to the treatment. 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 5 minutes apart. Extra attention was paid to ensure that the cornea was bathed with the drug by holding the animal horizontally for at least 1 minute after each instillation. Results from the two optometrists were averaged. Refractive error was expressed as the spherical equivalent (SE), that is, spherical error plus half of the cylinder error. No correction was made for the artifact of retinoscopy, which is relatively small in guinea pigs.[31]

Corneal curvature: The radius of the corneal curvature was measured with a custom-made infrared photokeratometer as described previously.[31,40] Readings were accepted only when the reflection of the light emitting diode (LED) rings was centered 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 dimension of the eye was determined 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. Results from 10 readings were averaged for each eye measured.

Data Presentation and Analysis

The results are presented as mean ± standard deviation (SD) unless otherwise stated. Paired *t*-tests were used to analyze the

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Table. Changes of Ocular Parameters With Time

Paradigms	Groups	Time Points	Refractive Error, D	Corneal Radius, mm	ACD, mm	LT, mm	VCD, mm	AL, mm
1: Without lenses	HiBS, $n = 24$	Baseline	3.68 ± 0.82	3.32 ± 0.07	1.12 ± 0.06	2.45 ± 0.12	3.42 ± 0.19	7.18 ± 0.11
		First week	4.90 ± 0.45	3.43 ± 0.11	1.11 ± 0.05	2.55 ± 0.14	3.55 ± 0.16	7.36 ± 0.12
		Second week	5.42 ± 0.39	3.52 ± 0.06	1.11 ± 0.04	2.64 ± 0.11	3.68 ± 0.13	7.50 ± 0.14
		Third week	5.84 ± 0.37	3.58 ± 0.07	1.12 ± 0.04	2.81 ± 0.09	3.82 ± 0.13	7.64 ± 0.11
		Change	2.17 ± 0.65	0.27 ± 0.07	0.00 ± 0.06	0.35 ± 0.10	0.40 ± 0.20	0.46 ± 0.14
	HiFL, $n = 20$	Baseline	3.69 ± 0.57	3.32 ± 0.07	1.10 ± 0.05	2.47 ± 0.15	3.44 ± 0.15	7.17 ± 0.10
		First week	4.81 ± 0.88	3.44 ± 0.11	1.12 ± 0.04	2.58 ± 0.10	3.57 ± 0.17	7.35 ± 0.15
		Second week	5.49 ± 0.70	3.50 ± 0.09	1.11 ± 0.05	2.67 ± 0.08	3.72 ± 0.16	7.44 ± 0.15
		Third week	5.95 ± 0.50	3.57 ± 0.09	1.12 ± 0.05	2.81 ± 0.10	3.84 ± 0.15	7.60 ± 0.18
		Change	2.26 ± 0.55	0.26 ± 0.05	0.02 ± 0.06	0.34 ± 0.12	0.40 ± 0.14	0.43 ± 0.21
	LoBS, $n = 22$	Baseline	3.69 ± 0.48	3.33 ± 0.06	1.10 ± 0.04	2.46 ± 0.14	3.42 ± 0.18	7.16 ± 0.10
		First week	4.64 ± 0.63	3.45 ± 0.07	1.12 ± 0.06	2.53 ± 0.11	3.57 ± 0.18	7.38 ± 0.11
		Second week	4.98 ± 0.55	3.51 ± 0.06	1.11 ± 0.05	2.63 ± 0.13	3.67 ± 0.25	7.49 ± 0.11
		Third week	5.10 ± 0.69	3.58 ± 0.50	1.12 ± 0.06	2.80 ± 0.11	3.86 ± 0.25	7.63 ± 0.14
		Change	1.40 ± 0.93	0.25 ± 0.06	0.03 ± 0.07	0.33 ± 0.15	0.44 ± 0.27	0.47 ± 0.18
	LoFL, $n = 20$	Baseline	3.69 ± 0.47	3.33 ± 0.07	1.10 ± 0.06	2.46 ± 0.11	3.42 ± 0.24	7.17 ± 0.08
		First week	4.56 ± 0.47	3.45 ± 0.07	1.12 ± 0.05	2.57 ± 0.19	3.56 ± 0.22	7.36 ± 0.17
		Second week	4.87 ± 0.57	3.52 ± 0.07	1.11 ± 0.06	2.63 ± 0.19	3.65 ± 0.26	7.48 ± 0.15
		Third week	5.08 ± 0.63	3.57 ± 0.06	1.10 ± 0.05	2.81 ± 0.11	3.84 ± 0.27	7.66 ± 0.10
		Change	1.39 ± 0.88	0.24 ± 0.05	0.00 ± 0.07	0.36 ± 0.13	0.42 ± 0.32	0.49 ± 0.12
2: With −4-D lenses	HiBS-L, $n = 22$	Baseline	3.69 ± 0.50	3.34 ± 0.08	1.09 ± 0.06	2.45 ± 0.11	3.46 ± 0.14	7.19 ± 0.11
		First week	3.05 ± 0.82	3.44 ± 0.06	1.11 ± 0.07	2.55 ± 0.15	3.71 ± 0.20	7.62 ± 0.11
		Second week	2.55 ± 1.03	3.52 ± 0.05	1.12 ± 0.06	2.67 ± 0.13	3.84 ± 0.17	7.69 ± 0.08
		Third week	2.24 ± 0.92	3.57 ± 0.05	1.11 ± 0.06	2.83 ± 0.11	3.98 ± 0.13	7.82 ± 0.10
		Change	−1.45 ± 0.99	0.23 ± 0.09	0.02 ± 0.04	0.38 ± 0.10	0.52 ± 0.16	0.64 ± 0.15
	HiFL-L, $n = 20$	Baseline	3.72 ± 0.61	3.32 ± 0.09	1.11 ± 0.05	2.48 ± 0.17	3.46 ± 0.17	7.19 ± 0.07
		First week	2.91 ± 0.99	3.44 ± 0.06	1.09 ± 0.05	2.54 ± 0.10	3.68 ± 0.17	7.58 ± 0.11
		Second week	2.37 ± 1.02	3.53 ± 0.07	1.10 ± 0.05	2.66 ± 0.14	3.86 ± 0.15	7.68 ± 0.08
		Third week	1.91 ± 0.93	3.59 ± 0.07	1.12 ± 0.07	2.82 ± 0.13	4.00 ± 0.14	7.80 ± 0.11
		Change	−1.81 ± 0.73	0.27 ± 0.09	0.02 ± 0.04	0.37 ± 0.16	0.54 ± 0.14	0.62 ± 0.14
	LoBS-L, $n = 24$	Baseline	3.72 ± 0.60	3.35 ± 0.05	1.09 ± 0.04	2.48 ± 0.15	3.48 ± 0.10	7.18 ± 0.12
		First week	2.73 ± 0.74	3.44 ± 0.06	1.13 ± 0.05	2.56 ± 0.14	3.72 ± 0.09	7.58 ± 0.14
		Second week	2.26 ± 0.81	3.52 ± 0.06	1.10 ± 0.05	2.69 ± 0.11	3.86 ± 0.08	7.73 ± 0.11
		Third week	1.42 ± 0.62	3.59 ± 0.06	1.09 ± 0.04	2.80 ± 0.13	4.03 ± 0.11	7.83 ± 0.10
		Change	−2.29 ± 0.50	0.24 ± 0.05	0.00 ± 0.03	0.33 ± 0.13	0.56 ± 0.15	0.65 ± 0.15
	LoFL-L, $n = 22$	Baseline	3.67 ± 0.60	3.33 ± 0.07	1.10 ± 0.06	2.48 ± 0.17	3.47 ± 0.12	7.19 ± 0.09
		First week	2.30 ± 0.58	3.43 ± 0.07	1.12 ± 0.06	2.57 ± 0.17	3.70 ± 0.16	7.56 ± 0.11
		Second week	1.84 ± 0.55	3.53 ± 0.06	1.13 ± 0.05	2.68 ± 0.11	3.85 ± 0.17	7.71 ± 0.16
		Third week	1.08 ± 0.48	3.57 ± 0.06	1.12 ± 0.06	2.82 ± 0.06	4.04 ± 0.16	7.81 ± 0.09
		Change	−2.58 ± 0.65	0.24 ± 0.07	0.02 ± 0.05	0.34 ± 0.15	0.57 ± 0.18	0.62 ± 0.12

ACD, anterior chamber depth; LT, lens thickness; VCD, vitreous chamber depth; AL, axial length. Data are presented as mean ± SD.

changes in ocular parameters between baseline and the end of the experiment for individual groups. As no interaction was found between spectral features and light intensity in either paradigm using factor analysis, the difference in changes between groups was compared by one-way ANOVA. If significant differences were detected, post hoc range tests were performed using the Duncan test. Additionally, unpaired *t*-tests were used to compare the means of independent groups with the same spectral composition but different intensities, or with different spectral features but the same light intensity. Pearson's correlation analysis was used to examine the relationship between the change of refractive error and that of axial length. All the statistical analysis was performed using SPSS 16.0 (SPSS, Chicago, IL, USA). The level for statistical significance was set at two-tailed 0.05.

Results

Data on all ocular parameters at different time points are shown in the Table. At baseline, none of the parameters were significantly different between groups. In addition, there was no significant difference between the right and left eyes (data not shown) in all groups for refractive error and axial parameters. Thus, all the results were based on data from the right eyes of the guinea pigs.

Refractive Error

There was a significant hyperopic shift in refractive error in all groups reared without lenses after 3 weeks of light exposure (Table). In contrast, the eyes of animals fitted with −4-D lenses developed a myopic shift (Table). Under both rearing conditions, unpaired *t*-tests indicated significant effects of light intensity but not spectral composition on the changes in refraction.

For guinea pigs reared without lenses, at the end of the experiment, refractive error in HiFL increased by 2.26 ± 0.55 D, followed by the HiBS (2.17 ± 0.65 D), LoBS (1.40 ± 0.93 D), and LoFL (1.39 ± 0.88 D) (one-way ANOVA: $F = 8.124$, $P < 0.001$). Post hoc analysis revealed that HiBS and HiFL belonged to one subset ($P < 0.05$), while LoBS and LoFL belonged to

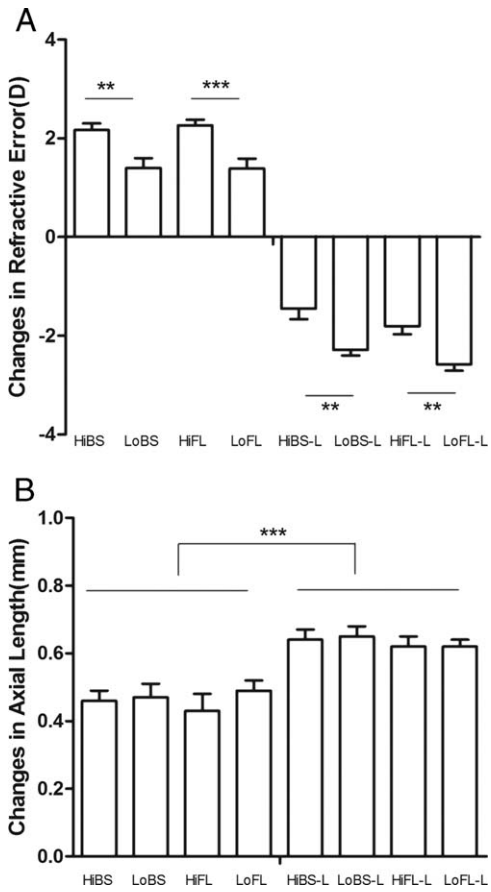

Figure 2. Comparison of the changes of refractive error (**A**) and axial length (**B**) among the groups. HiBS, high-intensity lighting of Solux halogen light; LoBS, low-intensity lighting of Solux halogen light; HiFL, high-intensity lighting of fluorescent light; LoFL, low-intensity lighting of fluorescent light; -L, with −4-D lenses; ACD, anterior chamber depth; LT, lens thickness; VCD, vitreous chamber depth; AL, axial length. Data are presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *Error bars*: ±SEM.

another subset ($P < 0.05$). When comparing different intensities in the same spectrum distributions, it was found that guinea pigs exposed to HiFL exhibited a significantly increased hyperopic shift compared to those exposed to LoFL (unpaired *t*-test: $t = 3.791$, $P < 0.001$; Fig. 2A). This was also true for HiBS and LoBS (unpaired *t*-test: $t = 3.239$, $P = 0.002$). Nevertheless, when comparing different spectrum distributions at the same intensity, there was no significant difference between HiBS and HiFL or between LoBS and LoFL (unpaired *t*-test: $t = 0.521$, $P = 0.605$ and $t = -0.056$, $P = 0.956$, respectively; Fig. 2A).

In contrast, for guinea pigs reared with lenses, LoFL-L had the greatest myopic shift of −2.58 ± 0.65 D, followed by LoBS-L (−2.29 ± 0.50 D), HiFL-L (−1.81 ± 0.73 D), and HiBS-L (−1.45 ± 0.99 D) (one-way ANOVA, $F = 8.804$, $P < 0.001$). Post hoc analysis revealed that, similar to guinea pigs reared without lenses, HiBS-L and HiFL-L belonged to one subset ($P < 0.05$) while LoBS-L and LoFL-L belonged to another subset ($P < 0.05$). Also similarly, when comparing different intensities in the same spectrum distributions, HiFL-L exhibited a significantly lower myopic shift when compared to LoFL-L (unpaired *t*-test: $t = 3.748$, $P = 0.001$). This was also true for HiBS-L and LoBS-L (unpaired *t*-test: $t = 3.584$, $P = 0.001$). However, when comparing different spectrum distributions at the same intensity, the differences in the myopic shift between HiBS-L and HiFL-L (unpaired *t*-test: $t = -1.405$, $P = 0.168$), LoBS-L and LoFL-L (unpaired *t*-test: $t = -1.038$, $P = 0.305$) were not statistically significant (Fig. 2A).

Corneal Curvature

The radius of corneal curvature increased significantly in all groups (paired *t*-test: all $P < 0.05$; see Table), with changes ranging from 0.23 to 0.27 mm. However, there was no significant difference in the changes between groups (one-way ANOVA: $F = 0.591$, $P = 0.623$ for groups without lenses, and $F = 0.988$, $P = 0.403$ for groups with lenses). This was also the case when data from groups without lenses and with lenses were pooled (one-way ANOVA: $F = 0.872$, $P = 0.53$).

Ocular Dimensions

The axial length of all groups increased throughout the experiment (Table). However, there was no significant difference among groups in guinea pigs reared either without lenses (one-way ANOVA: $F = 0.507$, $P = 0.678$) or with lenses (one-way ANOVA: $F = 0.212$, $P = 0.888$). When the data from all groups were pooled, it was found that axial elongation in guinea pigs reared with lenses was significantly greater than in those without lenses (unpaired *t*-test, $t = -7.92$, $P < 0.001$; see Fig. 2B).

Unlike the changes in refractive error, there was no statistically significant difference in axial elongation between the high and low lighting intensity with the same spectrum distribution (unpaired *t*-test: $t = -0.307$, $P = 0.760$ for Solux lamps, and $t = -0.113$, $P = 0.910$ for fluorescent lamps). Neither was there a difference between the Solux light and the fluorescent light with the same intensity (unpaired *t*-test: $t = -0.372$, $P = 0.712$ for high intensity, and $t = -0.67$, $P = 0.506$ for standard intensity).

The anterior chamber depth did not show a significant change during the observation period (paired *t*-test: all $P > 0.05$; see Table). In contrast, the thickness of the crystalline lens increased significantly with age in all groups (paired *t*-test: all $P < 0.05$). However, the changes in the thickness of the crystalline lens between groups were not statistically significant (one-way ANOVA: $F = 0.209$, $P = 0.89$ for groups without lenses, and $F = 0.763$, $P = 0.518$ for groups with lenses).

Correlation Between Changes in Axial Length and Refractive Error

Figure 3 shows the correlation between the changes of axial length and refractive error for the guinea pigs reared both without lenses (paradigm 1) and with lenses (paradigm 2). It is noted that the decrease of refractive error (i.e., more myopia) correlated significantly with the elongation of axial length for both paradigms ($R^2 = 0.550$ and 0.667; both $P < 0.001$), indicating that the refraction shift in both paradigms was largely axial in origin. The ratio of axial length elongation to the increase of myopia was also similar (−3.561 D/mm, 95% confidence interval [CI]: −4.260, −2.862) and (−4.599 D/mm, 95% CI −5.295, −3.902), respectively; $P > 0.05$). If the corneal flattening in both paradigms is considered, this ratio would increase to approximately 10 to 11 D/mm, as a 0.25-mm increase of corneal radius is equal to approximately a 6.5-D hyperopia shift (assuming that the refractive index of the cornea in guinea pigs is 1.3375).

Discussion

In the current study, we found that high-intensity lighting provided by either broad-spectrum lighting of the Solux

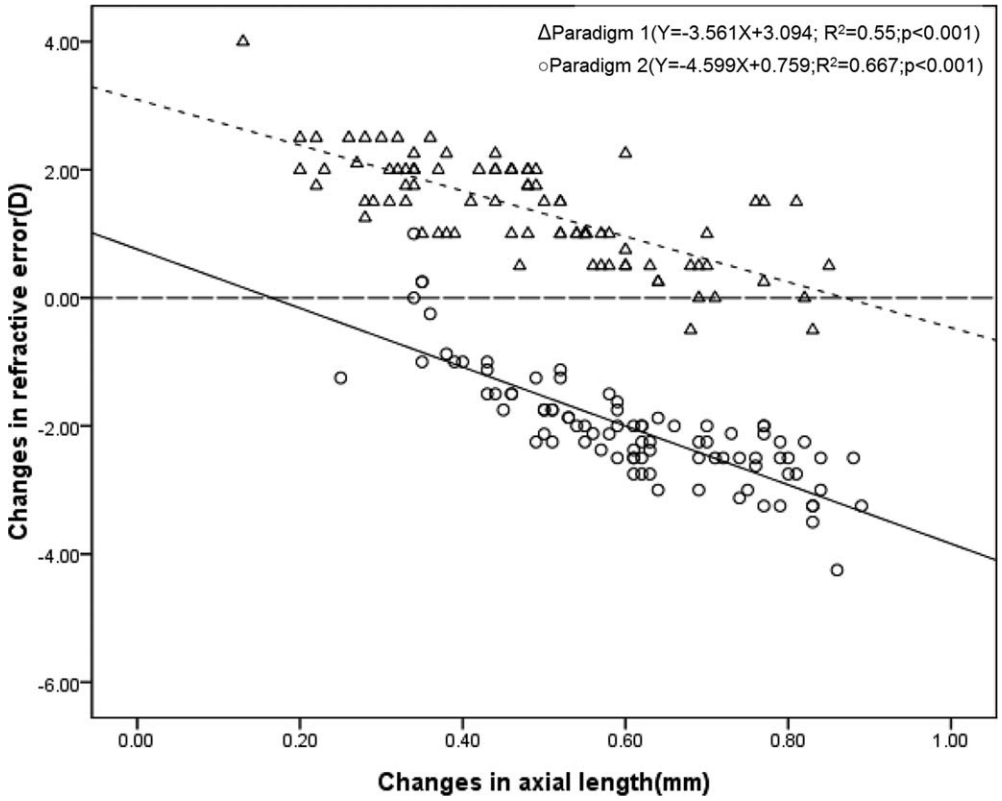

FIGURE 3. The correlations between changes of axial length and refractive error. *Triangles* represent the data from guinea pigs reared without lenses (paradigm 1), and *circles* represent the data from guinea pigs reared with lenses (paradigm 2). Both paradigms show a significant correlation between the changes of axial length and refractive error ($R^2 = 0.55$ and 0.667, respectively; both $P < 0.001$), indicating that the refraction shift in both paradigms was largely axial in origin.

halogen light or spiked-spectrum fluorescent light enhanced hyperopic shifts (guinea pigs reared without lenses) or retarded myopia development (guinea pigs reared with lenses), compared to low-intensity lighting. However, irrespective of light intensity, there was no difference in the effects of the two lamps.

In our results, one unexpected finding was the hyperopic shift in animals raised without lenses. This has not been reported in other studies.[31–33,39] There is no obvious explanation for the hyperopic shifts in normal refractive development in guinea pigs. It should also be noted that the biometric data in the present study do not match very well with those reported in previous studies[31–33,39]; however, data from these previous studies were also not consistent. In the present study, the longer the axial length, the more myopic shifts or less hyperopic the refractive error. However, actual myopia shifts were observed only in guinea pigs fitted with −4-D lenses. Apparently, the flattening of the cornea (Table) in animals reared without lenses had a greater effect than axial elongation, resulting in hyperopic shifts in 81 out of 86 animals after 3-week treatment. The different results we have obtained may be due to species differences. As for the axial length, the value found in the current study for the age-matched (1-month old) guinea pigs in the control group (LoFL) was 5.3% and 7.4% shorter, respectively, compared to the values from Zhou et al.[32] and Howlett and McFadden[31] (7.66 vs. 8.07 and 8.226 mm). The discrepancy could be related to the different ultrasound parameters used in the three studies. The frequency of ultrasound used in the current study was lower than in the experiments of Zhou et al.[32] and Howlett and McFadden[31] (10 vs. 11 MHz/20 MHz). The resolution and precision of the ultrasound used in this study were 0.01 and ±0.1 mm, respectively, while these parameters were not specified in the other two studies. These parameters may compromise the accuracy of axial length measurements and account for our failure to detect significant differences between groups, especially when the change during the experiment period was small. But the results on axial dimensions measured in the present study are still useful for the assessment of relative changes in axial components and in relation to the refractive error.

The protective effect of intensive illumination found in the present study was consistent with previous studies on other animals[13–16] (Siegwart JT, et al. *IOVS* 2012;53:ARVO E-Abstract 3457). One plausible theory for this effect is the dopamine-related pathway, as the release of dopamine from retinal dopaminergic amacrine cells is almost linear to the logarithm of the ambient lighting level,[13,41–44] and dopamine agonists inhibit experimental myopia in at least deprivation myopia.[45–48] The most convincing evidence for this hypothesis is the finding that the protective effect of bright light was abolished after a daily injection of spiperone (a dopamine D2 antagonist).[15] In addition, bright light was recently found to stimulate choroidal thickening.[49] Although there was some time delay (4 hours after the cessation of the bright light) and the magnitude was modest (+10% to +20%),[49] we speculate that choroidal thickening might also play a role in myopia inhibition by bright light exposure, as thicker choroids were linked to the inhibition of myopia.[50–52]

As mentioned previously, sunlight differs from common indoor lighting not only in illumination intensity, but also in the spectral composition and spectral distribution. In the only study comparing the myopia inhibition effect between sunlight and indoor lighting, it was shown that chicks exposed to

sunlight developed significantly less deprivation myopia than those exposed to indoor light (−1.1 ± 0.45 vs. −3.4 ± 0.6 D).[14] However, whether the greater effect of sunlight was associated with its UV component or with its stronger intensity was difficult to determine, since the halogen-quartz lamps used in that experiment were covered by UV-absorbing glass.[14] The Solux halogen lamp used in the current study emits radiation in the UV-A range (350–400 nm), which helped to clarify this puzzle. Our study showed that there was no significant difference in refractive change, at both 500 and 10,000 lux, between the UV-included Solux halogen lamps and the UV-free fluorescent lamps. Furthermore, in a study applying UV light with a high intensity (~200 lux, peaking at 390 nm with a half-band width of 25 nm), it was shown that UV did not affect emmetropization, and the chicken eyes compensated fully to the imposed negative lenses.[53] As chickens have UV cone photoreceptors,[54–56] this finding therefore showed that UV input from cone photoreceptors did not, at least at such intensity, counteract the myopigenic response induced by negative lenses. Thus, the present results indicate that inclusion of UV light in a polychromatic spectrum is unlikely to produce additional protection against myopia. It is not known if higher intensities of UV light would influence the compensation process or not, but the possible side effects of exposure to high-dose UV severely limit experimentation.

With regard to the spectral distribution, the Solux bulbs emit a smooth distribution of wavelengths, while the fluorescent light is composed of a spiked distribution. However, both of these have a broad spectral range. The brightness of the two light sources in the present study was made equivalent using a photometer, calibrated using the human L- and M-cone spectral sensitivity. The M-cone excitation was the same in the two conditions. Although the S-cone excitation was slightly different, it was unlikely to be substantially different, as both light sources have substantial energy at short wavelengths. Thus, both types of cones in guinea pigs may have been stimulated similarly, resulting in the same brightness of the two illuminants perceived by the guinea pigs, which was consistent with the similar refractive changes found in the current study. In other words, spectral distribution of polychromatic light does not seem to influence the inhibition effect against myopia by bright light, provided that the intensity is comparable.

It should also be pointed out that although the Solux halogen lamp mimics the sunlight spectrum propagated to the guinea pig retina, this lamp does not provide UV-B radiation (290–320 nm). Indeed, vitamin D_3, which has been postulated to influence scleral growth,[57] possibly by an effect on cell proliferation,[58] can be produced in the skin only with UV-B. Therefore, the lack of this range of radiation in the Solux halogen light prevents us from clarifying the issue of whether myopia is related to inadequate levels of vitamin D_3.

In conclusion, it is difficult, if not impossible, to provide a comprehensive test of the hypothesis that specific features of the spectral composition of light play an important role in the inhibition of the development of myopia by bright light, because there is effectively an infinite range of variations in spectral composition. We have therefore chosen to use two commonly used light sources in human environments with very different spectral compositions—one with a broad spectrum similar to that of sunlight, and one with a discontinuous and highly peaked distribution. There was no difference in refractive change in both natural development and compensation to negative lenses in guinea pigs reared with the two light sources. We cannot rule out the possibility that further research might find a particular pattern of spectral composition with particularly marked effects, but the current findings do not give any support to the idea that spectral composition plays an important role in the inhibition of experimental myopia. Nor do these experiments provide any support for a role of UV exposures. This supports the idea that the protective effects of bright light against the development of experimental myopia in animals depend primarily on the intensity of visible light, which also may apply to human myopia.

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