The Interactions Between Bright Light and Competing Defocus During Emmetropization in Chicks

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Purpose. The environment comprises multiple optical signals that affect eye growth. We aimed to determine if the inhibitory effects of myopic defocus and bright light (BL) against myopia are additive in the presence of the myopia-genic hyperopic defocus.

Methods. In experiment 1, three groups of 24 chicks each were fitted with the following multizone dual-power lenses (pl): pl/−10 D (50:50 area), +10/−10 D (50:50 area), and +10/−10 D (33:67 area) monocularly for 6 days. Half of each group were raised under normal illumination of 500 lux, 12/12-hour light/dark cycle, whereas the remainder were exposed to 6-hour BL of 40 klx and 6-hour 500 lux during the light cycle. In experiment 2, 38 chicks wore +10/−10 D (33:67 area) lenses monocularly for 8 days and were exposed to one of four light intensities for 6 hours per day—500 lux, 10 klx, 20 klx, or 40 klx—and received 500 lux for the remainder of the light cycle.

Results. In experiment 1, interocular difference in refractions after 6 days for the three groups were −3.6 D, +2.0 D, and −4.2 D, respectively, under normal light and were −0.9 D, +4.2 D, and +0.67 D under BL, manifesting as a shorter anterior segment and vitreous chamber. In experiment 2, the effect of BL increased with light intensity in the +10/−10 D (33:67) group, with a significant difference in refraction between the 10 klx and 20 klx groups (interocular difference −2.75 ± 2.76 D vs. 1.70 ± 2.40 D, $P < 0.01$), but plateaued between 20 klx and 40 klx (1.70 ± 2.40 D vs. 1.70 ± 0.35 D, $P > 0.05$).

Conclusions. The protective effects of myopic defocus and BL against experimental myopia were additive. The inhibitory effect of BL against myopia was dose dependent at 10 klx and above but plateaued at 20 klx.

Keywords: myopia, animal model, emmetropization, defocus, bright light

Myopia, also known as near-sightedness, is a condition of the eye in which images inaccurately focus in front of the photoreceptor layer of the retina. The extremely high prevalence of myopia in some East Asian populations has made this condition a focus of growing attention and concern.[1–5] The prevalence of myopia may be also increasing in Caucasian populations in Australia and the United States.[6–11] A variety of interventions have been used to control the progression of myopia (reviewed by Walline[12]). These include pharmacological interventions,[13] visual manipulations of bright light (BL), and optical defocus.[14–16]

BL has been proven to be effective in slowing the progression of myopia in animals[17–22] and probably in human too. Several animal studies have shown that BL (15 k to 40 klx), often 20 to 80 times higher than common indoor illumination (300∼500 lux), can suppress myopic eye growth in chicks, mice, and rhesus monkeys.[17–21] A number of recent studies have shown that children who spend more time outdoors have a more hyperopic refractive error and a lower incidence of juvenile-onset myopia.[22–26] In addition, a recent small-scale clinical trial provided preliminary evidence that elevated illumination in the classroom not only reduced the incidence of myopia but also slowed down refraction and axial length (AL) changes.[27] The protective effect of time spent outdoors against myopia does not seem to be mediated by physical exercise because indoor exercise does not inhibit myopia.[22]

Because the natural visual environment usually comprises a combination of optical signals,[28,29] the sign of defocus experienced by the retina is critical in determining refractive development. It has been observed that animals reared with full-field myopic defocus develop hyperopia, whereas hyperopic defocus results in myopia, indicating a locally bidirectional visual compensatory mechanism.[30] Importantly, myopic defocus was shown to dominate hyperopic defocus in modulating ocular refractive development.[31,32]

Our previous study has shown that myopic eye growth could be inhibited by applying myopic defocus using dual-power lenses and that the young animal eye was regulated by an equilibrium between the opposite hyperopic and myopic defocus.[28] The results indicated that chicken refractive error development was guided by a mechanism that integrates competing defocus stimuli simultaneously presented. For example, in our previous work when chickens were fitted with a full field +10 D/−10 D dual-power lens with 50:50 area ratio for the two power zones, the outcome was an intermediate refractive set-point slightly biased toward hyperopia. In guinea pigs, it was observed that incorporating a positive or plano power under a similar dual-power setting led to inhibited ocular growth and reduced myopia

compared to animals fitted with a single power lens having the same negative power.[29] Results from chicken and guinea pig studies indicated that retina integrates the sign and magnitude of competing defocus signals in a linear manner. In another study using primate model, marmosets monocularly treated with dual-power, multizone contact lenses of alternating powers (–5/+5 D, 50:50 area) have relative hyperopia in the treated eyes when compared with the single-vision control eyes. The amplitude of relative hyperopia was equivalent to that produced by a +5 D single-vision contact lenses.[33] In a different primate study, dual-power spectacles with 2-mm plano central zone and concentric alternating zones of +3 D and 0 D (+3 D/power lens [pl]) or –3 D and 0 D (–3 D/pl) were applied on the infant rhesus monkey. The resultant refractive errors in the +3 D/pl group were similar to that in the +3 D single-vision lens group, and the refractive errors in the –3 D/pl group was more hyperopic than that of the –3 D single-vision lens group.[34] Apparently, myopic defocus produced by the positive area on a 50:50 concentric multizone design dominated the direction of emmetropization in the primate models.

Soft contact lenses, introducing myopic defocus alongside with vision correction, were well tolerated by children and inhibited myopia progression in several clinical trials. There are two major categories in terms of lens design. The first category aims to introduce myopic defocus across the whole retina using concentric, multizone, dual-power lenses (similar to the lenses used in the present study). In a 20-month, two-phase, cross-over, clinical trial, Anstice and Phillips[35] found that full-time (13.15 ± 2.80 h/day) wear of a dual-power contact lens (2 D defocus) slowed myopia progression by 37%–54% in the first and second phases, respectively. In a 2-year, randomized, clinical trial, Lam et al.[36] found that part-time wear (6.46 ± 2.16 h/day) of a dual-power lens (2.5 D defocus) slowed myopia progression by 25%. Interestingly, the effectiveness of treatment increased with daily wearing time and plateaued at 60% in patients who wore the lens for ≥8 h/day. The second category of design aims to introduce relative myopic defocus mainly on the peripheral retina. Walline et al.[37] reported that a center-distance multifocal contact lens with progressive plus power toward the periphery could slow myopia progression by about 50%, and another study by Sankaridurg et al.[38] reported a 34% effectiveness. To summarize, clinical trials using relative myopic defocus to retard myopia progression in children were partially successful with effectiveness ranging between 30% to 60%, depending on the lens design and wearing schedule.[12] Interestingly, the dominating influence of myopic defocus on emmetropization observed in animals[32,34] were not directly translated in the clinical setting, suggesting that myopic defocus alone may not be able to fully stop myopia progression in children. Thus, an emerging research question is whether multiple optical signals may combine to further their effect in controlling myopia.

In this study, we aimed to explore how BL interacts with myopic defocus under the influence of myopia-genic hyperopic defocus. In particular, we hoped to establish if myopic defocus and BL could be combined to increase the inhibition of myopic eye growth and to determine the optimal intensity of BL.

Methods

All animal experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and all the experimental procedures were approved by The Hong Kong Polytechnic University's Animal Ethics Committee. Specific pathogen-free eggs of white leghorn chicks were obtained from Jinan Poultry Co. Ltd. (Jinan, China). Newborn chicks were obtained by incubating the fertilized eggs for 21 days in the Centralized Animal Facilities of The Hong Kong Polytechnic University (Hung Hom, Hong Kong, China).

Lighting

The chicks were reared under a 12:12 hour light/dark cycle, with lights providing general fluorescent illumination of 500 lux switched on between 7 AM and 7 PM, until experiments started at 5 days old. An additional light source, BL with intensities of 40 klx, 20 klx, or 10 klx was applied only to the treatment groups for 6 hours between 10 AM to 4 PM using broad-spectrum (350–680 nm) metal halide lamps (Philips HPI-T 250W; Phillips Lighting Luminaires [Shanghai] Company, Shanghai, China) in a RVP 350 housing. These lamps illuminated the cages from above at different distances calibrated using a hand-held photometer positioned at the level of the animal.

Dual-Power Lenses

The 5-day old chicks were monocularly treated with lenses for 6 to 8 days, which were attached to the right eyes via a pair of Velcro rings (generic) that facilitated removal and reattachment for regular measurements and daily cleaning. The left eyes remained untreated and served as a control. Refractive errors and biometric parameters, including AL, vitreous chamber depth (VCD), anterior chamber depth (ACD), lens thickness, and choroidal thickness (CT) were measured by retinoscopy and high-frequency A-scan ultrasonography, respectively, every 2 days. The details of these procedures have been previously described.[28,39]

Concentric, multizone, dual-power lenses, designed based on the Fresnel's principle, were manufactured from PMMA cast molding by the State Key Laboratory of Ultraprecision Machining Technology in the Polytechnic University (Hung Hom, Hong Kong, China). All lenses had an optical zone diameter of 11 mm and an anterior radius of curvature of 6.68 mm. The following three designs of dual-power lenses were used: (1) the 50:50 area pl/–10 lens, in which each –10 D annulus is coupled with one plano annulus; (2) the 50:50 area +10/–10 lens, in which each +10 D annulus is coupled with one –10 D annulus; and (3) the 33:67 area +10/–10 lens, in which each +10 D annulus is coupled with two consecutive –10 D annuli. The central zones of the lenses have a –10 D power. The pitch width of the annulus was 0.4 mm for the pl/–10 lens and was 0.1 mm for the other two lenses. The multizone dual power lenses (Fig. 1D) produced two distinct image planes (Fig. 1E). When dual-power lenses were applied at the beginning of the experiment, the –10 D area on the lens produced a hyperopic defocus on the retina, whereas the remaining lens area produced a myopic defocus (+10 D) or a focused image (plano) on the retina according to the second lens power. The multizone design incorporated alternating annuli of different powers throughout the lens from center to periphery and provided a relatively stable ratio between the two powers independent of eye movements or changes in pupil size under different illumination (Fig. 1). Pupil sizes of animals as measured using a photoreceptor (PowerRefractor, Multi-Channel System, Steinbeis Transfer Center, Germany) were 2.77 ± 0.31 mm ($n = 3$) and 2.38 ± 0.12 mm ($n = 3$) under normal and bright lights (40 klx), respectively. Fig. 1 A through C show the frontal view of the three dual-power lenses and how pupil constrictions may affect the effective ratios of the two lens powers theoretically. For the pl/–10 D (50:50) lens, the ratio between plano and minus powers is approximately 1 under CL and may decrease to about 0.5 when the pupil constricts (Fig. 1A). For the +10/–10 D (50:50) lens, the plus/minus ratio does not undergo any unidirectional change

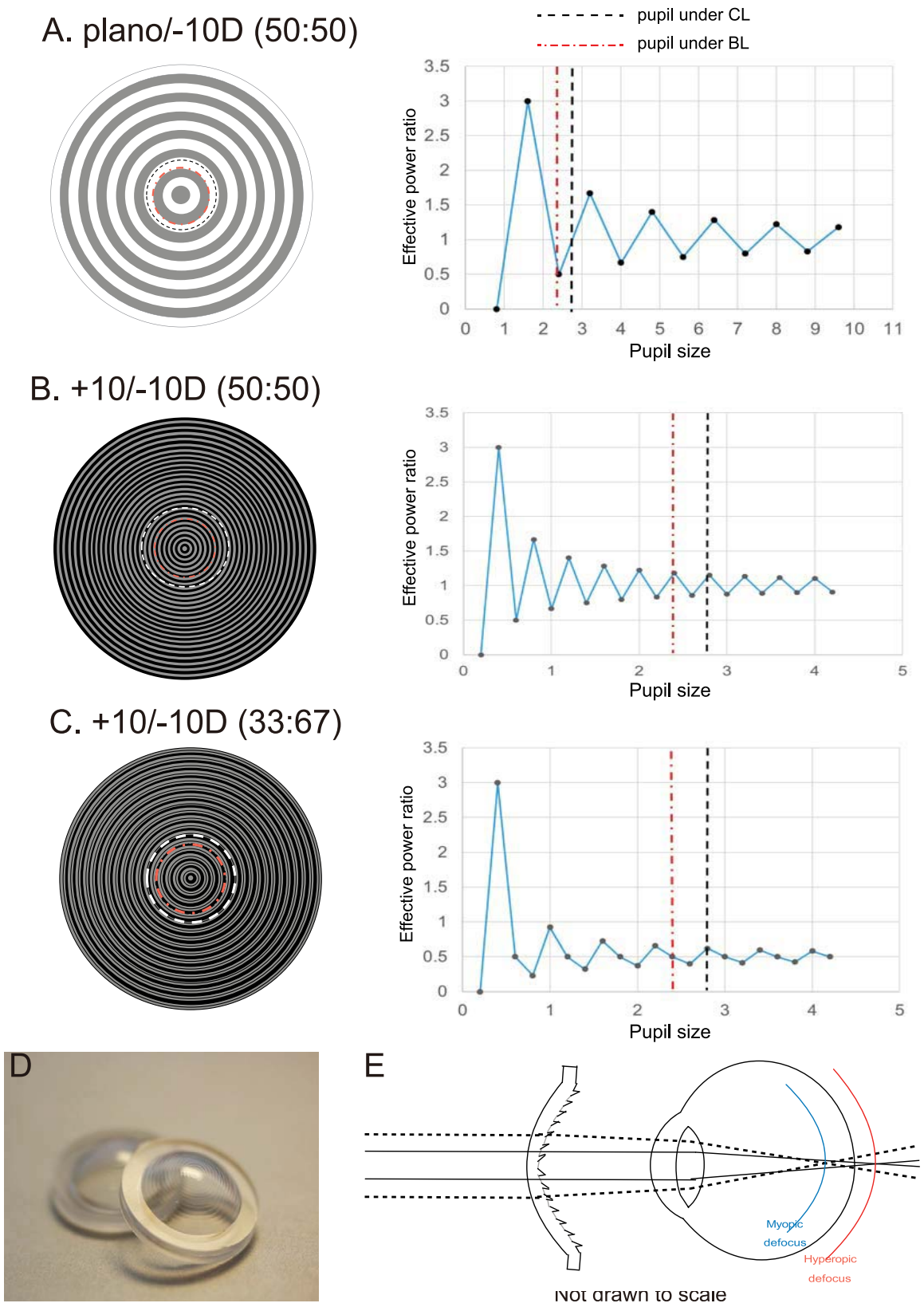

Figure 1. (**A**–**C**) Schematic diagrams showing how the annuli on the lenses were arranged to provide different powers and ratios between powers. *White annuli*, plano; *gray annuli*, −10 D; *black annuli*, +10 D. (**A**) *Left panel*: each plano annulus is coupled with one −10 D annulus. *Right panel*: the average power ratio is 1, which varies slightly with changes in pupil size. (**B**) *Left panel*: each +10 D annulus is coupled with −10 D annulus. *Right panel*: the average power ratio is 1, which varies minimally with changes in pupil size. (**C**) *Left panel*: each +10 D annulus is coupled with two consecutive −10 D annuli. *Right panel*: the average power ratio is 0.5, which varies minimally with changes in pupil size. (**D**) Photograph of concentric dual-power lenses. (**E**) Diagrammatic representation of the two focal planes induced by a +10/10 D dual-power Fresnel lens in the chicken eye.

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Table. Summary of Experimental Conditions in Different Groups

Experiment	Lens Type	Duration of Lens Wear, Day	Setting of Lighting	*n*
Experiment 1: ocular responses to competing defocus and bright light	pl/−10 D (50:50)	6	40k for 6 hours, 500 for 6 hours	12
	pl/−10 D (50:50)	6	500 for 12 hours	12
	+10/−10 D (50:50)	6	40k for 6 hours, 500 for 6 hours	12
	+10/−10 D (50:50)	6	500 for 12 hours	12
	+10/−10 D (33:67)	6	40k for 6 hours, 500 for 6 hours	12
	+10/−10 D (33:67)	6	500 for 12 hours	12
Experiment 2: effect of different illumination of bright light	+10/−10 D (33:67)	8	40k for 6 hours, 500 for 6 hours	10
	+10/−10 D (33:67)	8	20k for 6 hours, 500 for 6 hours	10
	+10/−10 D (33:67)	8	10k for 6 hours, 500 for 6 hours	8
	+10/−10 D (33:67)	8	500 for 12 hours	10

when the pupil constricts. Instead, it varies within a small range between 0.85 and 1.15 (Fig. 1B). For the +10/−10 D (33:67) lens, the plus/minus ratio varies between 0.39 and 0.59 and does not undergo any unidirectional change when the pupil constricts (Fig. 1C). In summary, pupil constriction would either lead to little change in the relative ratio of defocus or would lead to a slightly increased ratio of hyperopic defocus.

Experimental Design

Experiment 1. The Interaction of BL and Competing Defocus on Emmetropization Set Point. Chicks were treated monocularly with one of the dual-power lenses for 6 days. Chicks were reared under either normal laboratory illuminance (500 lux) throughout the light cycle or with 6-hour BL (40 klx; $n = 12$) and 6-hour normal illuminance during the treatment period. A summary of grouping and experimental conditions is shown in the Table.

Experiment 2. The Dose-Response Effect of BL on Defocus-Induced Emmetropization. A total of 38 chicks were monocularly treated with a +10/−10 (33:67) lens for a period of 8 days. Of these chicks, 28 were kept under BL at different illuminations of 40 klx ($n = 10$), 20 klx ($n = 10$), and 10 klx ($n = 8$) for 6 hours per day and under normal laboratory illuminance (500 lux) for the remaining period of the light phase. A total of 10 chicks receiving 12-hour normal illumination served as controls. The +10/−10 (33:67) lens was chosen because strongest effects were observed in chicks with this lens when combined BL of 40 klx in experiment 1.

Statistics

Ocular refraction and biometric measures data were expressed as means ± SD of interocular difference (IOD) for each group. A two-way ANOVA followed by Sidak correction of Bonferroni inequality for multiple testing was used to analyze ocular parameters between groups.

Results

Experiment 1

The BL of 40 klx produced hyperopic biases in refractive error (Rx) in all three dual-power lens groups. As shown in Figure 2, the chicks ($n = 12$) treated with pl/−10 D (50:50) lens developed myopia of −3.58 ± 1.02 D under normal lighting (500 lux). In contrast, the daily exposure to BL (40 klx) for a period of 6 hours per day induced a relatively mild myopic refraction of −0.92 ± 0.67 D ($P < 0.01$). This intergroup difference in refraction was reflected by a correspondingly shorter IOD of AL in the BL group than in the control group (0.07 ± 0.11 mm vs. 0.27 ± 0.07 mm, $P < 0.01$). In particular, the IOD of VCD and ACD in the BL group were significantly shorter than those in the control group (0.05 ± 0.13 mm vs. 0.15 ± 0.09 mm, $P < 0.05$; 0.03 ± 0.06 mm vs. 0.17 ± 0.08 mm, $P < 0.01$).

As expected, the introduction of myopic defocus in the +10/−10 D (50:50) group induced a relatively hyperopic bias under control lighting when compared with that in the pl/−10 D (50:50) group (see Fig. 3). The exposure to BL made the refraction of this group even more hyperopic (4.2 ± 1.4 D vs. 2.0 ± 0.7 D, $P < 0.01$). The BL group had a shorter IOD in ACD (−0.10 ± 0.04 mm vs. 0.01 ± 0.03 mm, $P < 0.01$), VCD (−0.21 ± 0.10 mm vs. −0.08 ± 0.09 mm, $P < 0.01$), and a resultant IOD of AL (−0.26 ± 0.11 mm vs. −0.12 ± 0.09 mm, $P < 0.01$).

Decreasing the area ratio of myopic defocus in the +10/−10 D lens to 33% resulted in a myopic emmetropization set point under normal lighting (Fig. 4). Intriguingly, the application of BL induced a mild hyperopic shift in refraction (0.67 ± 0.9D vs. −4.2 ± 2.7D, $P < 0.01$). This difference was attributable to the shorter AL (−0.03 ± 0.10 mm vs. 0.28 ± 0.20 mm, $P < 0.01$), ACD (−0.01 ± 0.05 mm vs. 0.12 ± 0.13 mm, $P < 0.01$), and VCD (−0.03 ± 0.13 mm vs. 0.15 ± 0.15 mm, $P < 0.01$). The IOD of CT in the BL group was statistically thicker than that in the CL group at day 2 (22.67 ± 42.11 μm vs. −67.83 ± 69.54 μm, $P < 0.01$). There was no significant difference in the IOD of lens thickness between the CL and BL group (data not shown).

Experiment 2

As shown in Figure 5, the strength of illuminance determined the direction of emmetropization under competing defocus (+10/−10 D) at the 33:67 ratio. Application of BL at all three levels (40 klx, 20 klx, and 10 klx) resulted in significantly shorter and more hyperopic eyes than those of the control group. At day 6 after treatment, the ALs and ACDs of the three BL groups were significantly shorter than the CL group. The statistical differences were sustained and extended to day 8, when the VCD was also significantly shorter in the BL group.

Interestingly, on day 8 both the AL and VCD of the 10 klx group differed significantly from those of the other BL groups. The AL in 10 klx, although shorter than the control light group (0.11 ± 0.17 mm vs. 0.46 ± 0.17 mm, $P < 0.01$), was longer than that of the 40 klx (0.11 ± 0.17 mm vs. −0.14 ± 0.15 mm, $P < 0.01$) and 20 klx (0.11 ± 0.17 mm vs. −0.06 ± 0.15 mm, $P < 0.05$) groups. A similar pattern was observed for the VCD. These findings indicated a general dose-response trend, with a saturation effect above 20 klx, as there was no significant difference between 20 klx and 40 klx. This finding is consistent with the results of previous studies,[39] which showed that the antimyopia effect of BL was dose dependent.

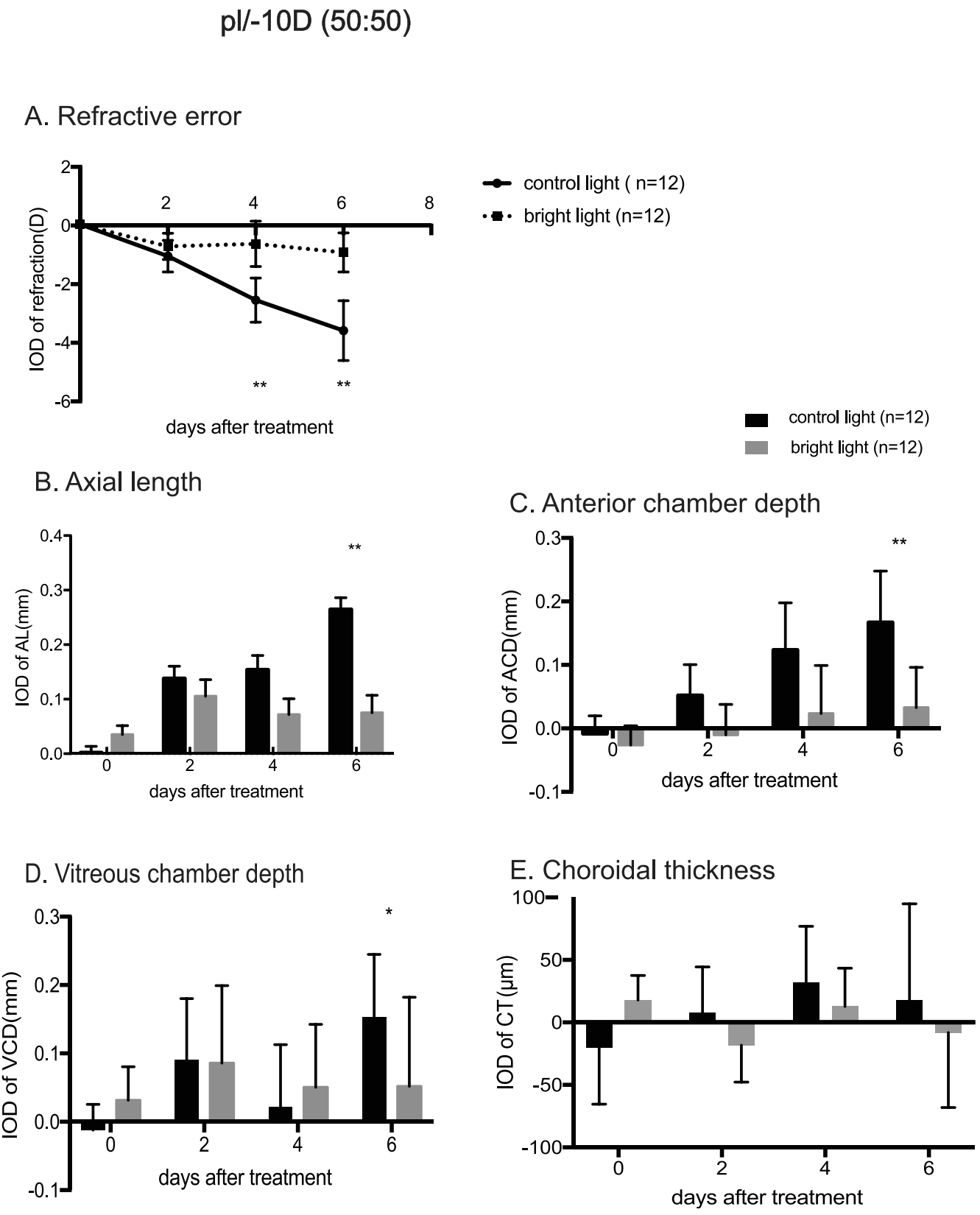

FIGURE 2. The interocular differences (treated eye minus control eye) in (**A**) refractive error, (**B**) AL, (**C**) ACD, (**D**) VCD, and (**E**) CT after 6 days of treatment with pl/−10 lens. *Black solid line* and *black bars* show the mean values of control light (500 lux) animals. *Dashed line* and *gray bars* show the mean values of bright light (40 klx) animals. *Error bars* represent SD. $^{*}P < 0.05$. $^{**}P < 0.01$. The refractive error and certain biometric data in the bright light group were significantly different from those of control light group at day 6.

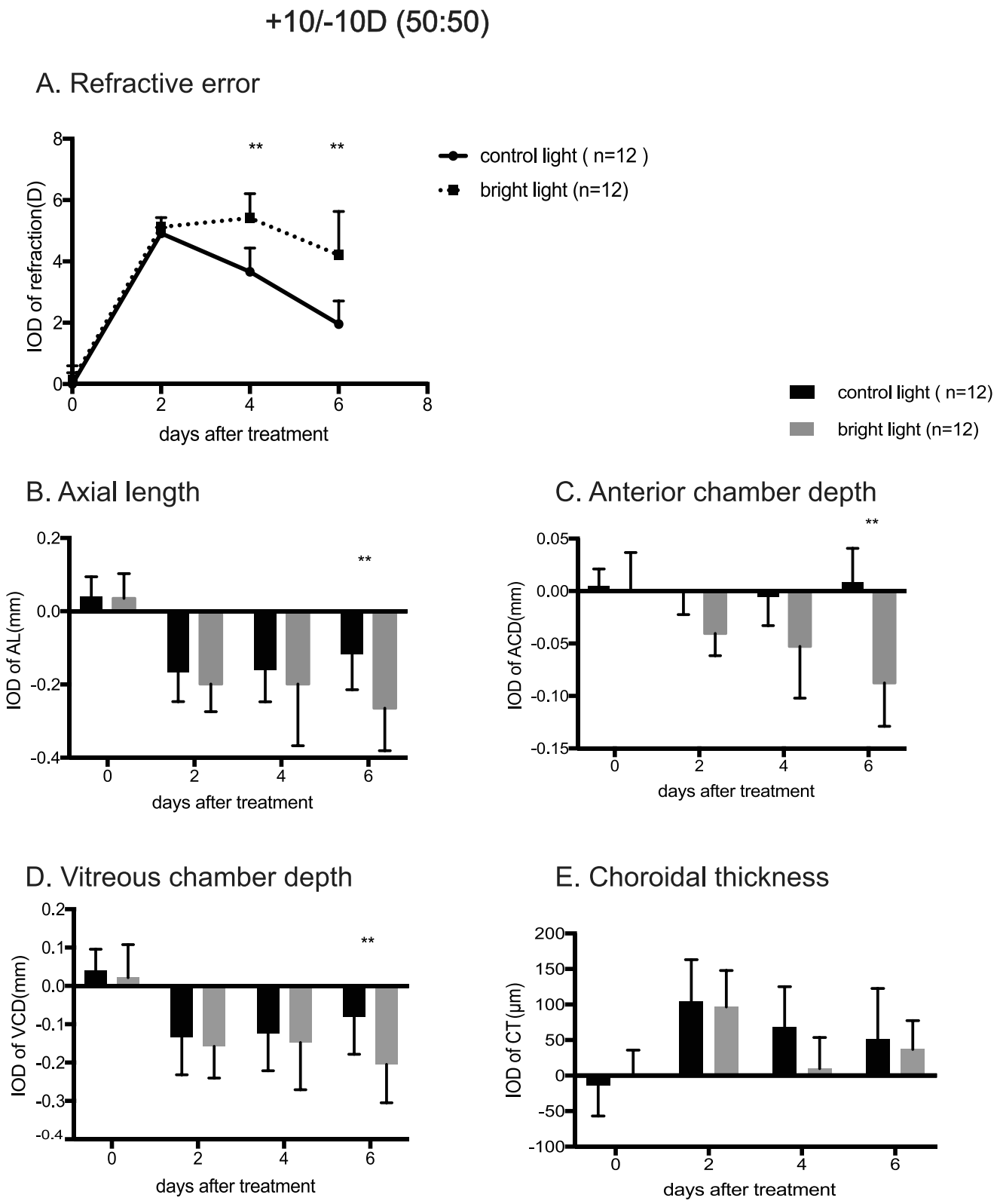

FIGURE 3. The interocular differences (treated eye minus control eye) in (**A**) refractive error, (**B**) AL, (**C**) ACD, (**D**) VCD, and (**E**) CT after 6 days of treatment with +10/−10 (50:50) lens. *Black solid line* and *black bars* show the mean values of control light (500 lux) animals. *Dashed line* and *gray bars* show the mean values of bright light (40 klx) animals. *Error bars* represent SD. $^{*}P < 0.05$. $^{**}P < 0.01$. Bright light (40 klx) produced a significantly hyperopic shift in chicks.

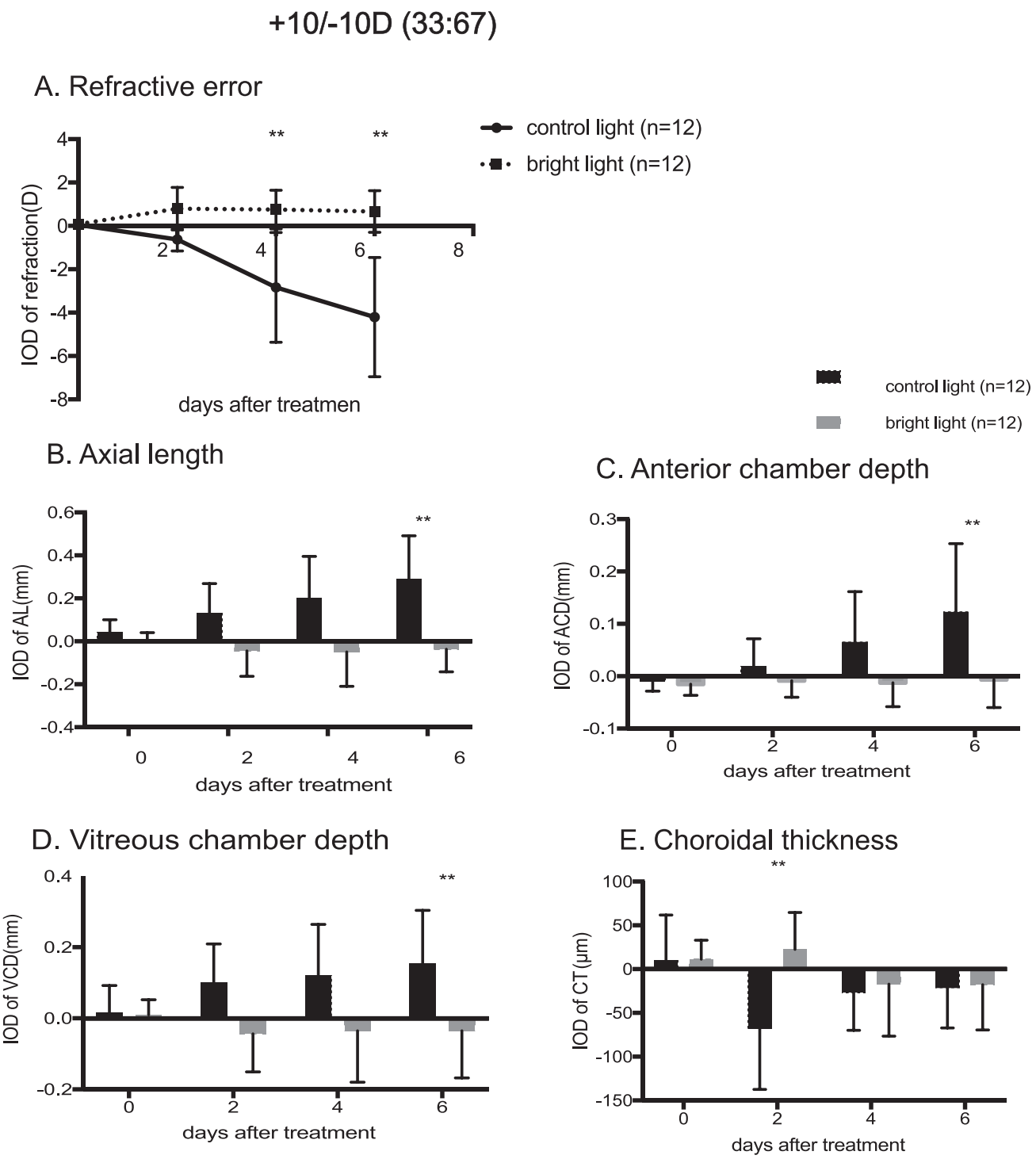

FIGURE 4. The interocular differences (treated eye minus control eye) in (**A**) refractive error, (**B**) AL, (**C**) ACD, (**D**) VCD, and (**E**) CT after 6 days of treatment with +10/−10 (33:67) lens. *Black solid line* and *black bars* show the mean values of control light (500 lux) animals. *Dashed line* and *gray bars* show the mean values of bright light (40 klx) animals. *Error bars* represent SD. $^{*}P < 0.05$. $^{**}P < 0.01$. The bright light (40 klx) produced significantly hyperopic shift on chicks wearing +10/−10 (33:67) lens.

The CT under the three BL conditions were significantly different from that of control light at day 2 (10 klx vs. control: 11.87 ± 46.97 μm vs. −58.38 ± 59.02 μm, $P < 0.05$; 20 klx vs. control: 29.28 ± 63.83 μm vs. −58.38 ± 59.02 μm, $P < 0.01$; 40 klx vs. control: 26.10 ± 45.60 μm vs. −58.38 ± 59.02 μm, $P < 0.01$).

DISCUSSION

Integrated Ocular Response Toward Competing Defocus

It has been established that ocular growth is sensitive to optical defocus, with ocular growth being accelerated by hyperopic

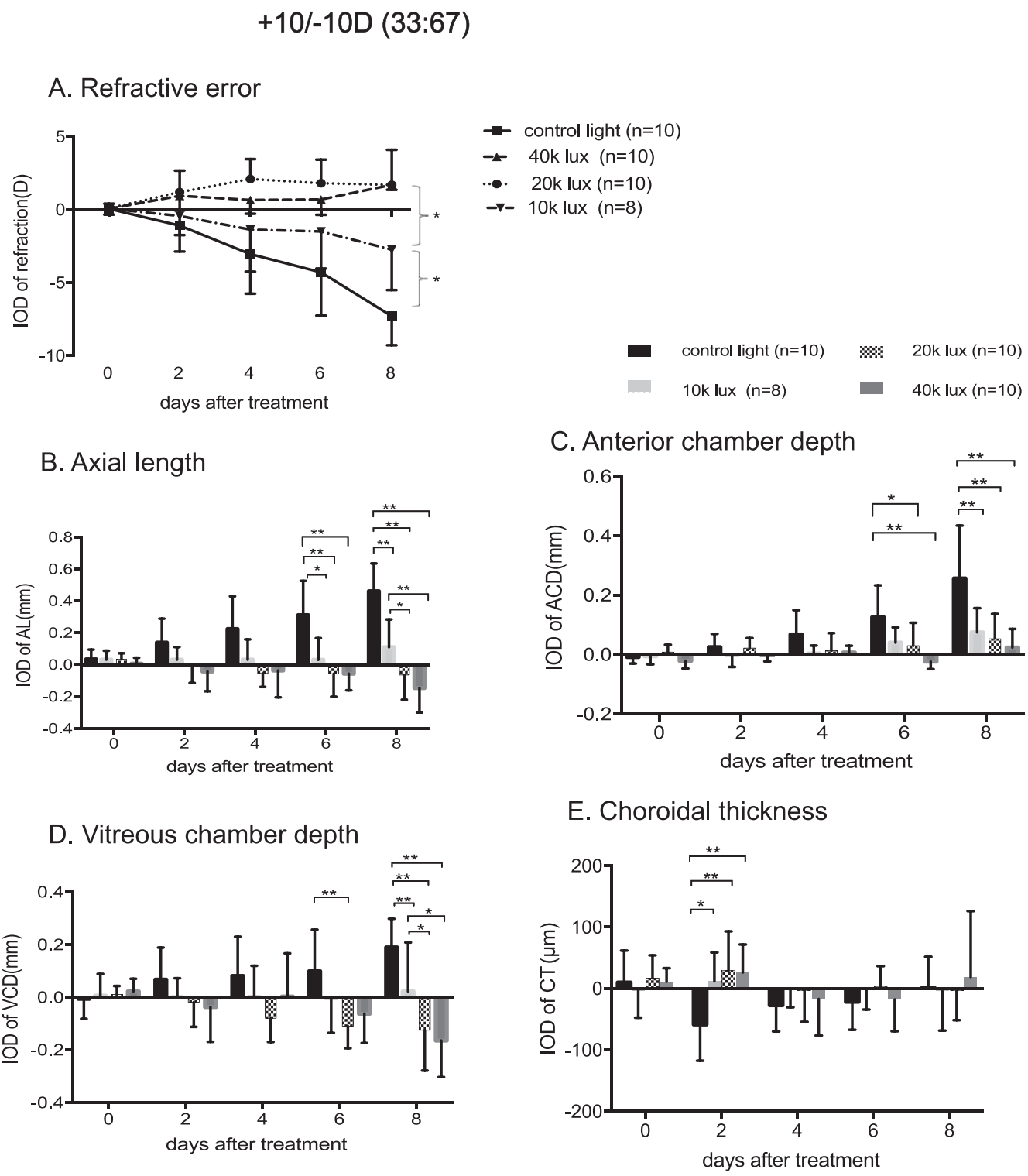

Figure 5. The interocular differences (treated eye minus control eye) in (**A**) refractive error, (**B**) AL, (**C**) ACD, (**D**) VCD, and (**E**) CT during 8 days of monocular +10/−10 (33:67) lens wear under light intensity of 10 klx, 20 klx, 40 klx, and control light. *Error bars* represent SD. $^{*}P < 0.05$. $^{**}P < 0.01$. Bright light of 40 klx and 20 klx inhibited myopia induced by the lens by a similar extent. Bright light of 10 klx showed a smaller, but still significant inhibition. The CT in the three bright light groups were significantly greater than those of the control light group on day 2 only.

defocus and slowed by myopic defocus.[40,41] Previous studies, including our own work, have found that the set point of emmetropization could be modulated by the magnitude and ratio of the applied opposing defocuses.[28,29,32–34,42] Using a chick model, our previous study showed that the refractive set point of the treated eye lay between the two optical powers of the concentric dual-power lens. After 6 days of treatment with dual power lenses of +20/−10, +10/−10, +5/−10, and 0/−10 D, the resultant refractive errors were +13.5, +4.7, −0.6, and −3.9 D, respectively.[28] The resultant refractive error became increasingly myopic when the ratio of hyperopic defocus was increased through increased area ratio of −10 D power in a

+10/−10 D lens. The interocular difference in refractive error was +4.7, −6.7, and −9.3 D for ratios of 50:50, 33:67, and 25:75, respectively. In a recent study on the monkey, it was shown that even when the more positive-powered zones made up only a fraction of the dual-focus lens surface area, refractive development was still strongly influenced by relative myopic defocus.[32] These findings suggest that a mechanism capable of integrating spatially competing defocus signals is present in the retina and is robust across species and that myopic defocus introduced across the visual field is a strong "stop" signals to slow eye growth.

When compared with a conventional lens-induced paradigm, this dual-power lens paradigm provides a platform to study the complex interaction between the "go" signal and the "stop" signal, allowing researchers to titrate the relative contribution of individual stimuli and study emmetropization near its equilibrium state.

The Effect of BL on Defocus-Induced Emmetropization

It has been reported that daily exposure to BL of 15 klx for 5-hour/day alone could slow the progression of lens-induced (−7 D) myopia and speed up the progression of lens-induced (+7 D) hyperopia in chicks without changing the final set point after 5 days.[18]

In contrast, the current study has demonstrated that BL not only slowed the progression of lens-induced ametropia but also altered the set points of emmetropization under all three competing defocus conditions produced by the three dual-power lenses in our present study (experiment 1). Daily exposure to BL for 6 hours produced a hyperopic shift in refractive error and reduced responding myopic change of axial dimensions. Theoretically, such a shift could result if pupil constriction increases the contribution of the relative positive lens power. However, our calculation in the method section (Fig. 1) has ruled out this possibility. A possible explanation for this new finding is that the weaker "stop" signal of BL may be masked when stronger "go" or "stop" signals, such as high power hyperopic or myopic defocus, are present. Thus, its effect on emmetropization becomes determining only when the other stimuli are balanced and the emmetropization system is near an equilibrium state.

Summated "Stop" Effects and the Dose-Response Effect

The interaction of myopic defocus and BL was demonstrated in the results from experiment 1 (see Fig. 6), in which the mean IOD Rx in the pl/−10 D (50:50) group under control light and BL was −3.58 D and −0.92 D, respectively. This indicates that BL alone produced a hyperopic shift of +2.66 D under the 50% −10 D "go" signal. Under control lighting, IOD Rx after 6 days of pl/−10 D (50:50) and +10/−10 D (50:50) treatments were −3.58 D and 1.9 D, respectively. Because both lenses were composed of −10 D power in half of their optical area, the relative hyperopic shift of 5.5 D in the latter group resulted from the presence of the 50% +10 D "stop" signal produced by the other half of the lens. The refractive error was most hyperopic (5.5 D) when both "stop" signals were present in the group of +10/−10 (50:50) under the BL condition, with a hyperopic shift of 7.78 D. Therefore, the "stop" effects from myopic defocus and BL were additive.

To investigate the dose-response effect of BL under competing defocus, in the second experiment, BL exposures of 20 klx and 10 klx were used in addition to 40 klx. The +10/−10 D (33:67) dual-power lens was selected because it had induced the largest intergroup difference in Rx between the BL and control groups in experiment 1. Figure 5 showed that rearing chicks under BL of 40 klx for 6 hours per day, for a period of 8 days, significantly altered the refractive error, making it more hyperopic than that under the control light (1.70 ± 0.35 D vs. −7.30 ± 1.99 D, $P < 0.01$). After 8 days of treatment under 20 klx, the IOD refractive error was 1.70 ± 2.40 D, which was not significantly different from that 40 klx group. However, the 20 klx group displayed significantly more hyperopia than the 10 klx group, although the refractive error of the 10 klx group was statistically more hyperopic than the control light group. Therefore, it was concluded that the antimyopia effect of BL under our competing defocus condition was dose dependent. A similar dose-response effect has been reported by Ashby et al.,[17] in which the protective effect of diffuser removal under 15 klx was significantly enhanced if the diffusers were removed under a higher illumination level of 30 klx. Our study has shown that BL illuminance of even 10 klx could lead to a partial, but significant, inhibition against myopic changes under competing defocus conditions.

Biometric Changes in Response to BL

Ashby et al.[17] suggested that the protective effect of BL against axial elongation manifests specifically in the VCD and that there were no changes in ACD or the corneal radius of curvature induced by BL over normal light levels. In contrast, we found that the ACD of chicks under BL was significantly shorter than those reared under control light in all three dual-power lens groups. Because the IOD of lens thickness did not differ significantly between the lenses, we postulate that the corneal curvature may have been flattened by BL. Further work is required to characterize any changes in the cornea.

A well-known feature of emmetropization is that the choroid responds to myopic defocus and hyperopic defocus by increasing and decreasing its thickness, respectively. Thus, the choroid also plays a role in the modulation of ocular elongation in response to defocus.[43] The rapid, but transient, changes in thickness occur within hours following introduction of defocus and at least 24 hours prior to measurable changes in AL growth.[44,45] In our study, the IOD of CT under all three high illumination conditions were significantly thicker than that of the controls after 2 days treatment with +10/−10 D (33:67) lens (Fig. 5E). There is an apparent dosing effect, as the IOD in CT in the 20 klx and 40 klx groups were greater than that of the 10 klx group; however, this dose-response relationship is inconclusive because the differences did not reach statistical significance.[44]

Lan et al.[46] found that chicks exposed daily to 15 klx BL with no lens treatment had slightly more thickened choroid than controls kept under normal light, indicating that BL exposure alone induced choroidal thickening. In our study, the chicks exposed to opposing defocus using +10/−10 D (33:67) lenses had a 67-μm thinner choroid in the treated eye when compared with the contralateral eye when raised under normal light. Interestingly, such choroidal thinning was reversed when chicks were raised under BL. The increased CT of the lens-treated eyes in the three BL groups when compared with the contralateral eyes ranged from 11∼29 μm. It appears that BL increased the CT of the defocus-treated eye to a greater extent than that of the control eye, suggesting that BL may be interacting with myopic defocus to produce a stronger thickening effect on choroid.

Similar to the choroidal thickening induced by myopic defocus,[47] thickening induced by BL in our study was also transient. The exact molecular mechanism regulating CT and the mechanism for the transient nature of the change is unclear. It has been suggested that dopamine and nitric oxide are key

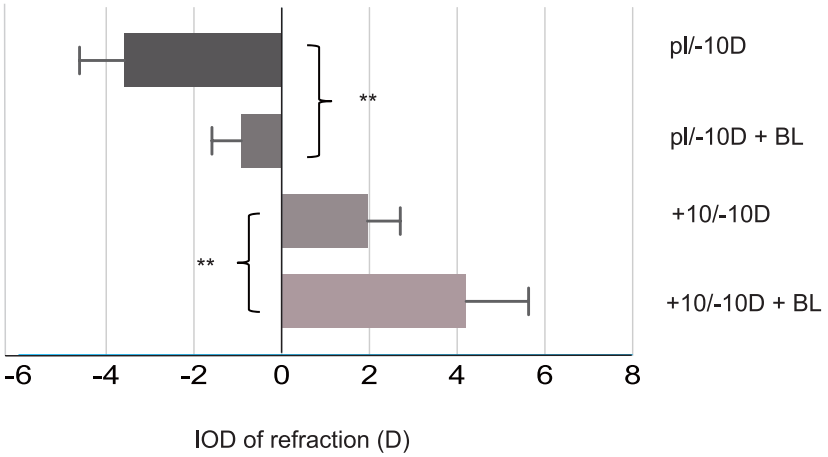

FIGURE 6. The additive effect of myopic defocus and bright light under hyperopic defocus. Interocular differences of refraction in the four groups applied with two different lenses and two different lightings. *Error bars* represent SD. $^{**}P < 0.01$.

neurotransmitters that mediate the signals regulating choroidal response.[48,49]

Possible Mechanisms Underlying the Summated Effect of Myopia Defocus and Bright Light

It is known that dopamine is one of the retinal neurotransmitters involved in the signaling cascade underlying the optical regulation of eye growth. Furthermore, it is a major "stop" signal mediating the protective effect of bright light against myopia. This is supported by animal experiments showing that dopamine D2 antagonist, spiperone, could abolish the protective effect of bright light against form-deprivation myopia[18] and could prevent the ocular growth inhibition normally effected by the brief periods of vision in form-deprived eyes.[50] Dopamine is normally released from dopaminergic amacrine cells that receive inputs from multiple cone pathways including intrinsically photosensitive retinal ganglion cells,[51] which are less sensitive to light compared to photoreceptors. It was postulated that dopaminergic activation increases with increased inputs from both the photoreceptor/bipolar-cell pathway and the intrinsically photosensitive retinal ganglion cells pathway when illuminance is high.[52]

The exact mechanism through which the retina decodes the directional defocus signals remains elusive. It is possible that myopic defocus and bright light target separate neuronal pathways, which independently process directional blur and brightness visual information in parallel at an early stage[53] before they are integrated as a combined signal to modulate ocular growth at a later stage. This is supported by the fact that retinal dopamine release following the application of defocusing lenses correlates with the magnitude, but not the sign of defocus.[54] Also, spiperone did not prevent the ocular growth inhibition normally effected by the brief periods of vision in negative lens-wearing eyes.[50]

In the present study, myopic defocus and BL produced an additive effect against the myopia-genic effect of hyperopic defocus. This finding may indicate that simultaneously applying two "stop" signals through the parallel neuronal pathways in the early stage of retinal processing could maximize the downstream combined "stop" signal.

Clinical Implications

The natural visual environment comprises visual signals of positive and negative defocus as well as information for brightness.[28,55] Our results showed that both myopic defocus and BL are important factors in the inhibition of excessive eye growth induced by hyperopic defocus. The ultimate goal for myopia research is to devise an intervention that stops or even reverses myopia progression in humans. There are currently several regimens known to be partially effective in inhibiting myopia progression, including defocus-based contact lenses and spectacle lenses, orthokeratology, atropine, and BL. However, they are limited in efficacy when applied alone (slowing progression by 30%–70%). These results suggest that tackling one factor at a time is not an ample solution, being inadequate to counteract the "go" signals that induce myopic eye growth. It is a reasonable strategy to combine two or more interventions to achieve extra protection against myopia progression. The results from the present study suggest that it is worthwhile to test the combination of defocus-based interventions with BL treatment for children with the hope that it may halt or substantially reduce their myopia progression.

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