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Light intensity modulates corneal power and refraction in the chick eye exposed to continuous light

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ABSTRACT

Continuous exposure of chicks to light was shown to result in severe hyperopia, accompanied by anterior segment changes, such as severe corneal flattening. Since rearing chicks in complete darkness results only in mild hyperopia and minor changes in corneal curvature, we hypothesized that light intensity may play a role in the development of refractive changes under continuous light illumination. To test this hypothesis, we examined the effects of rearing chicks under various continuous light intensities. More specifically, we investigated the refractive parameters of the chicks' eyes, and avoided light cycling effects on ocular development. To this end, thirty-eight chicks were reared under 24-h incandescent illumination, at three different light intensities: 10,000 lux ($n = 13$), 500 lux ($n = 12$), and 50 lux ($n = 13$). Their eyes underwent repeated retinoscopy, keratometry, and ultrasound biometry, as well as caliper measurements of enucleated eyes. Both refraction and corneal refractive power were found to be correlated with light intensity. On day 90 after hatching, exposure to light intensities of 10 000, 500, and 50 lux resulted in hyperopia of +11.97 ± 3.7 (mean ± SD) +7.9 ± 4.08 and +0.63 ± 3.61 diopters (D), respectively. Under those intensities, corneal refractive power was 46.10 ± 3.62, 49.72 ± 4.16, and 56.88 ± 4.92 D, respectively. Axial length did not differ significantly among the groups. The vitreous chamber was significantly deeper in the high than in the low-intensity groups. Thus, during the early life of chicks exposed to continuous lighting, light intensity affects the vitreous chamber depth as well as the anterior segment parameters, most notably the cornea. The higher the intensity, the more severe was the corneal flattening observed and the hyperopia that developed, whereas continuous illumination at low intensities resulted in emmetropia. Thus, light intensity is an important factor that should be taken into account when studying refractive development.

1. Introduction

Refractive development is dependent on visual experience such as image defocus and lighting conditions. Chicks that have been deprived of form vision by occluding their eyes via lid suturing (Raviola & Wiesel, 1978), translucent diffuser (Wallman, Gottlieb, Rajaram, & Fugate-Wentzek, 1987), or lens (Irving, Sivak, & Callender, 1992; Schaeffel, Glasser, & Howland, 1988; Schmid & Wildsoet, 1996b; Sivak et al., 1990) become ametropic. Illumination parameters such as photoperiod (Stone, Lin, Desai, & Capehart, 1995; Troilo, Li, Glasser, & Howland, 1995) and light intensity (Harrison, Bercovitz, & Leary, 1968) affect postnatal chicks' eye growth in a complex pattern.

The effects of continuous light on ocular parameters have been examined in various animals (Bartmann, Schaeffel, Hagel, & Zrenner, 1994; Li, Troilo, Glasser, & Howland, 1995; Liu et al., 2004; Smith, Bradley, Fernandes, Hung, & Boothe, 2001; Stone et al., 1995; Zadnik et al., 2000). In chicks, interrupting normal diurnal lighting rhythms by continuous lighting disrupts the emmetropization process and results in severe hyperopia. Ocular changes reported under these conditions include reduced corneal curvature, shallowing of the anterior chamber, increased intraocular pressure, enlarged axial length, and deepening of the vitreous chamber (Lauber & Oishi, 1987; Li et al., 1995; Stone et al., 1995). In mature rats, 19 days of exposure to continuous light resulted in a myopic shift, an effect that was attributed partially to corneal steepening and not to axial changes (Zadnik et al., 2000). In primates, it was found that exposing newborns for half a year to continuous light resulted in "unusual emmetropization" (Smith et al., 2001) in three out of nine monkeys. Two monkeys developed axial anisometropia and one manifested a myopic error. The study concluded that the variations from the expected developmental sequence observed in three monkeys may reflect individual differences. However, the authors also raised the possibility that aspects of the emmetropization process may not operate

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as effectively under constant light as they do under ordinary rearing (Smith et al., 2001). Thus, although continuous light results in a marked effect on the refractive development of vertebrates, its effect on refractive error in monkeys was not fully established.

Studies on the effect of continuous light on the growth of chick eyes were carried out under a wide range of light intensities, from only several to thousands of lux. After 80 days of continuous light exposure under 700 lux of fluorescent light, the induced hyperopia was more than +15 D (Li et al., 1995), whereas dark rearing (zero light intensity) induced milder ocular changes and hyperopia that varied from +3.11 to +8.24 D (Gottlieb, Fugate-Wentzek, & Wallman, 1987; Guyton, Greene, & Scholz, 1989; Troilo et al., 1995; Yinon & Koslowe, 1986). It seems that since the light intensity varied among the studies, the induced hyperopia was different. Liu et al. (2004) studied the development of emmetropization under constant light with relatively dim intensities of 0.3, 33, 133, and 500 lux. Only the latter intensity was identified as having a degree of hyperopia that was statistically different from the refractions of light/dark-reared controls (Liu et al., 2004). Thus, under continuous light, intensity might be a covariant for the development of refractive error.

We studied the effect of a wide range of light intensities on chicks' refractive development, corneal curvature, corneal thickness and diameter, anterior chamber depth, lens thickness and diameter, vitreous chamber depth, equatorial diameter, and axial length. In order to examine the effects of particular intensities, in all our experiments, we masked the effect of the photoperiod by continuous illumination.

2. Methods

2.1. Animals and their rearing conditions

Thirty-eight newly hatched White Leghorn female chicks (Hemed Farms, Israel) were raised in temperature-controlled cages via continuous air circulation (days 17, 33 ± 0.5 °C; days 790, 23 ± 1 °C). The chicks were supplied with food and water ad libitum. The experiment and animal handling were approved by the Animal Welfare Commission of Tel Aviv University and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. During the first 7 days after hatching, the chicks were kept in a cage (120 × 60 × 60 cm) with lighting according to a 10-h/14-h light/dark cycle of incandescent light with an intensity of 500 lux.

Seven days after hatching, the chicks were subjected to baseline measurements, and were then divided randomly into three groups. Each group was placed in a 2.3 × 1.7 × 4 m cage with constant illumination until the end of the experiment.

The chicks were exposed to incandescent light at three different levels of light intensity. Group 1, the high-intensity group ($n = 13$), was raised under bright light with an intensity of ~10,000 lux; group 2, the intermediate-intensity group ($n = 12$), was raised under moderate lighting of ~500 lux; and group 3, the low-intensity group ($n = 13$), had dim light with an intensity of ~50 lux. The illumination of the cages was standardized only to white light bulbs (fluorescent light bulbs radiate a different light spectrum from that of incandescent lamps) that were placed 2 meters above the floor level. The cages of the low and medium intensity groups were illuminated with one bulb of 5 watts and 40 watts, respectively. The cage of the high-intensity group was illuminated with four 100-watt bulbs at each corner, and one 300-watt bulb at the center.

The highest light intensity chosen, 10,000 lux, was equivalent to the intensity outdoors at noon on a sunny day, which can reach thousands of lux. The lowest, 50 lux, is an intensity at which chicks can carry on normal activity. Light intensity was measured at floor level at the center of the cage, using a calibrated Megatron Spectroradiometer (Megatron, London, UK).

2.2. Optical measurements

In all three groups, optical measurements were carried out while the chicks were anesthetized at 7, 30, 60, and 90 days after hatching. Subcutaneous xylazine solution 2%, 5 mg/kg and ketamine, 20 mg/kg, were administered for anesthesia. Cycloplegic ocular refraction was assessed using a Nikon Streak Retinoscope. Binocular cycloplegia was induced with eye drops containing 0.1% vecuronium bromide (Schwahn & Schaeffel, 1994). The refractive state was determined at a 66-cm working distance, using lens bars to neutralize the two principal meridians. Refraction was expressed as spherical equivalents (sphere ± cylinder/2).

For keratometry, we used a calibrated Javal-Schiotz (Haag-Streit) keratometer and calculated the mean of the two meridians. Because the cornea of the newly hatched chick is steep, we extended the measuring range of the instrument by adding convex lenses (+1.25 to +6 D). A correction for the true radius of the cornea was made on the basis of measuring the apparent radii of metal balls of known radii (range 3.95–9.55 mm) through these convex lenses.

For ultrasound biometry, we used a calibrated Allergan Humphrey ultrasound biometer (model 820) equipped with a tonometer-mounted, hard tip probe, operated in the manual mode. The mean of three to five measurements of axial length was taken. On day 90 after hatching, we determined the means of 3–5 in vivo measurements of the vitreous chamber depth, anterior chamber depth, lens thickness, and corneal thickness using an A-mode ultrasound device (EchoScan US-1800; Nidek, Fremont, CA), and an ultrasound pachymeter (Paxis; Biovision), for the latter. Corneal thickness measurements, obtained with BVI ultrasound pachymetry, were found to be highly repeatable (Gunvant, Broadway, & Watkins, 2003). The mean limbus-to-limbus corneal diameter along the 180° and 90° meridians was calculated from measurements made on day 90, using a calibrated manual micrometer.

Following the optical and ultrasound examinations, the chicks were euthanized with pentobarbitone sodium (60 mg/kg, i.v.). Their eyes were enucleated and the equatorial diameter and lens thickness were measured immediately afterwards, using a calibrated micrometer, and the average values of the horizontal and vertical meridians were calculated.

2.3. Data analysis

Data are reported as means ± SD. Means of the optical measurements of the eyes and ocular components were evaluated by one-way analysis of variance (ANOVA) for comparison within and among the groups. Post hoc pair-wise multiple comparisons were made using Dunnett's t-test for unequal variances. Pearson analysis was used to correlate among refraction, corneal power, and light-intensity exposure. A regression line depicts corneal power as a function of refraction. For statistical analysis of the results, we used the SigmaStat program (version 12, SPSS, Inc., Chicago, IL). Differences of $P < .05$ were considered statistically significant.

3. Results

3.1. Refraction

Baseline measurements obtained on day 7 regarding refraction, keratometry, and axial length showed no differences among the