The Effects of Low Intensity Ambient Lighting on Refractive Development in Rhesus Monkeys (Macacca mulatta)

by

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ABSTRACT

PURPOSE: Elevated ambient lighting levels protected animals from certain forms of experimental myopia, suggesting that alterations in ambient lighting levels influence refractive development. The purpose of the studies reported in this dissertation was to evaluate the extent to which low ambient lighting influences refractive development in primates and to determine whether and how low ambient lighting levels cause myopias.

METHODS: Infant rhesus monkeys (Macaca mulatta) were reared under reduced or "dim" ambient lighting (~50 lux) with either unrestricted vision or one of the monocular lens treatments that induces experimental anisometropias. The development of their refractive errors, corneal powers, and ocular axial dimensions was measured longitudinally and compared to those in monkeys reared under typical laboratory lighting ("normal" light) with the same visual conditions. Their choroidal thickness changes were also longitudinally measured to reflect the activity of refractive regulation.

RESULTS: The results showed that (1) dim light did not produce myopia in monkeys reared with unrestricted vision, but increased the variability in refractive error and reduced the likelihood of successful emmetropization; (2) dim light did not increase nor reduce the magnitude of form-deprivation myopia (FDM), but interfered with the refractive development after the discontinuation of form-deprivation and reduced the probability of recovery form FDM; (3) dim light reduced the probability of lens-induced compensating changes, increased the variability in refractive development, and reduced the degree of compensating anisometropias. All refractive observations were associated with alterations in vitreous chamber depth. The failures in developing the anticipated vision-induced anisometropias were associated with an absence of vision-induced relative choroidal thickness changes.

CONCLUSIONS: Dim light is not necessarily myopiagenic; however, extended exposure to dim light could cause myopia through reductions in the efficacy of visual mechanisms that normally regulate refractive development.

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Chapter 1.

General Introduction

Common myopia (short-sightedness) is a refractive disorder marked by excessive elongation in the vitreous chamber in comparison to the total optical power of the eye. Myopia not only causes visual impairment when uncorrected, but also increases the risk of vision-threatening ocular complications. The prevalence of myopia has increased in the recent decades and is estimated to reach a 50% global prevalence by 2050, imposing a significant and increasing burden to public health (for a review, see: Holden *et al.*, 2016).

Despite the high and increasing prevalence of myopia and its associations with severe, irreversible vision loss, treatment and prevention for this condition have not been very successful due to its etiological complexity. It was proposed that myopia develops because external environmental and/or behavioral factors disrupts the homeostasis of eye growth, which is actively regulated by vision-dependent mechanisms (Wallman and Winawer, 2004; Flitcroft, 2013; Troilo *et al.*, 2019). Active visual regulation is firstly supported by the systematic changes in refractive error and in the reductions in the inter-individual variability of refractive error that are associated with normal refractive development, or "emmetropization" (for the inquiry related to this phenomenon, see Chapter 2). This age-associated refractive error change is dependent on visual experience, because animals that are deprived of normal vision fail to exhibit normal age-associated refractive error changes and frequently develop myopia (form-deprivation myopia, or FDM) (Raviola and Wiesel, 1985; Barathi *et al.*, 2008; Howlett and McFadden, 2006; Smith III and Hung, 2000; Siegwart Jr. and Norton, 1998; Troilo and Judge, 1993; Wallman, Turkel, and Trachtman, 1978), a condition that could be at least partially reversed if normal visual experience is timely restored (for the inquiry related to this

phenomenon, see Chapter 3). Animal studies further showed that animals reared with ophthalmic lenses could alter their ocular growth rate to eliminate the lens-imposed optical errors (Barathi *et al.*, 2008; Hung *et al.*, 1995; Norton *et al.*, 2010; Schaeffel *et al.*, 1988) (for the inquiry related to this phenomenon, see Chapter 4). Together, this evidence indicates that visual experience, particularly the eyes' refractive state, regulates the rate of axial ocular growth.

Other environmental factors can also influence refractive development and myopia. Among them, ambient lighting levels have been suggested to contribute to the protective effect of more outdoor time in reducing the risk of myopia in children (Dirani *et al.*, 2009; French, Ashby, Morgan, & Rose, 2013; Guggenheim *et al.*, 2012; Jones *et al.*, 2012; Rose *et al.*, 2008; Wu *et al.*, 2013). In comparison to the laboratory lighting levels employed in most experiments (on the order of hundreds of lux), elevated ambient light levels have been found to consistently reduce the magnitude of FDM (Siegwart Jr., Ward, and Norton, 2012; Ashby, Ohlendorf, and Schaeffel, 2009; Smith III, Hung, and Huang, 2012; Karouta and Ashby, 2015; Chen *et al.*, 2017). In addition, in some species, elevated ambient lighting levels also slowed the emmetropization-related reductions in hyperopias (Cohen *et al.*, 2011, Cohen *et al.*, 2012) and decelerated the axial compensations to negative lenses (Ashby *et al.*, 2010, Norton and Siegwart Jr., 2013). These findings suggest that higher lighting levels might be protective against myopia. Conversely, depending on how the mechanism that mediates these protective effects operates, reduced ambient lighting levels might be associated with the onset and/or development of myopia. The purpose of the research reported in the present dissertation is to evaluate the refractive effects of reduced ambient lighting and to evaluate the extent to which reduced ambient lighting poses a risk of myopiagenesis.

General methods

Subjects

Infant rhesus monkeys (Macacca mulatta) were employed as the primary subjects. The experiments were conducted on animals because the proposed interventions cannot be applied in humans. Rhesus monkeys were the optimal animal model for these experiments due to the similarity in their refractive development and visual physiology with humans (see Chapter 2).

Main Intervention

The main intervention for all experiments was reduced ambient lighting ("dim light" or DL), which was produced by fitting an aluminum deposited, polyester film closely to the ceiling light panels. In order to facilitate between-study comparison (Cohen *et al.*, 2011, 2012; Ashby *et al.*, 2009), the target ambient illuminance level was ~50 lux as measured at levels between the upper and lower cages (waist-level). The spectral characteristic of this experimental dim ambient lighting is described in the Method sections of Chapters 2. At approximately 3 weeks of age, the subjects were randomly assigned to one of the four vision-treatment groups (see below) and reared under dim light until ~1 year of age.

The control condition for dim light was typical, or "normal" laboratory illumination ("normal light" or NL) that is commonly employed in our primate housing areas. The mean ± standard deviation of ambient lighting intensity = 504 ± 168 lux (correlated color temperature = 3170K). All of the control animals were reared under this "normal" lighting conditions.

Vision-induced ametropia

Except for one of the dim light groups in which subjects were reared with unrestricted vision, all dim-light subjects were reared with one of the three treatment lenses starting from the onset of the dim light rearing period until approximately 150 days of age. The treatment lenses consist of either a diffuser, a +3 diopter (D) lens, or a -3D lens in front of the treated eyes and a zero-power lens in front of the fellow control eyes. In previous studies, these lens paradigms consistently induced axial anisometropias in infant monkeys reared under normal ambient lighting (Hung *et al.*, 1995; Smith III & Hung, 2000).

Outcome measures and data collection

The primary outcome measure was refractive error, which was measured using cycloplegic retinoscopy and was presented as the spherical-equivalent power of the spectacle-plane correction. It is known that the eye's refractive error is regulated by local mechanisms that operate on signals derived from visual experience, and that the refractive errors in the two eyes of a given animal are largely independent. This independence allows interocular differences in refractive error to be employed as a more sensitive measure of the effects of monocular lens-wear on refractive development.

The explanatory outcome measures were the ocular parameters, including corneal power, conventional ocular axial dimensions (anterior chamber depth, lens thickness, vitreous chamber depth), and sub-foveal choroidal thickness. Corneal power was measured using hand-held keratometer or video topographer. Ocular axial dimensions were measured using A-scan ultrasonography. For details of these measurements, see the Method sections of Chapters 2-4.

The constellations of ocular axial dimensions and corneal powers determine the nature of refractive error, which is essential in order to relate any refractive findings (especially any myopic changes) to the development of ametropias in humans. Specifically, the common form

of human myopia is primarily attributed to the axial elongation in the vitreous chamber (i.e. axial myopia). For infant monkeys reared under normal ambient lighting, the between-individual variability in refractive development associated with unrestricted vision is primarily determined by variations in vitreous chamber elongation. In addition, the refractive errors induced by defocusing lenses are also associated with difference in vitreous chamber depth (Qiao-Grider *et al.*, 2010). Evaluation of the axial dimension changes for dim light subjects could reveal the ocular components that are affected by dim light. This is potentially important because, at least in some species, ambient lighting properties and/or lens rearing has also been shown to alter the development of other ocular optical components (e.g., corneal power) (Li and Howland, 2003; Cohen *et al.*, 2008; Li *et al.*, 1995; Cohen *et al.*, 2011). In this respect, an alternative measure for the role of ocular growth in refraction is the vitreous chamber to corneal radius-ratio (VC/CR ratio), which can partially account for the potential influence of inter-subject differences in corneal power on the expected changes in refractive error and vitreous chamber depth. If corneal power development conform with the typical course of development and the refractive errors are primarily determined by vitreous chamber depth, a cross-sectional examination should reveal a linear relationship between VC/CR ratio and refractive error. Correlational analyses based on VC/CR ratios provide sensitive means for the examination of the axial nature of refractive errors even when no significant between-group or between-eye difference in refractive error/vitreous chamber depth is present (see Chapter 2 and Chapter 4).

Sub-foveal choroidal thickness is defined as the distance between Bruch's membrane and the outer choroidal border along the normal to Bruch's membrane. It was measured from cross-sectional images acquired using spectral-domain optical coherence tomography (SD-OCT) segmented using a customized Matlab program (MathWorks, Natick, MA, USA). The average choroidal thickness of a 300 μm horizontal region that is centered at the deepest point of the macula depression was reported. Previous studies have shown that visual experience that

induces refractive changes usually also induces relative choroidal thickness changes that are in the appropriate directions (Troilo, Nickla, and Wildsoet, 2000a; Wallman *et al.*, 1995; Wildsoet and Wallman, 1995). Specifically, monocular form-deprivation (Wallman *et al.*,, 1995; Troilo, Nickla, and Wildsoet, 2000b) and imposed hyperopic defocus (Hung *et al.*, 2000; Wildsoet & Wallman, 1995) typically cause relative choroidal thinning in the treated eye in comparison to the untreated control eye. Depending on the magnitude of change in relation to the eye size, the refractive implications of these changes vary between species. For infant rhesus monkeys, the observed relative choroidal thickening and thinning does not cause significant refractive alterations; however, due to their consistent presence and constant relationship with the nature of visual stimulation, they can be used as a predictor for the upcoming refractive changes (Hung *et al.*, 2000) and thus a surrogate observation that reflects whether the visual stimulation (form-deprivation and imposed defocus) had produced functional signals that alter the rate of axial elongation.

Control data

Control data were obtained from four groups of infant monkeys previously reared under normal ambient lighting without visual restriction (the NL controls) or with the same visual restrictions that correspond to the specific experiment (normal light form-deprivation, lens-induced myopia, and lens-induced hyperopia, or NL-FD, NL-LIM, and NL-LIH). A large subset of these data has been published and discussed previously (e.g. Hung, Arumugam, She, *et al.*, 2018). The rearing and data acquisition procedures for the NL-monkeys were similar to those for the DL-monkeys.

Statistical analysis

The longitudinal development of refractive error and its interocular difference, as well as some ocular parameters, were compared using mixed-effect model analyses (Rabe-Hesketh and Skrondal, 2012). The dependent variables were specified as a 2^{nd} order polynomial function of age to reflect the curvilinear nature of their normal development. In comparison to the repeated-measures ANOVA method employed in previous non-human primate studies, mixed-effect model is advantageous in that it does not depend on the assumption that the variances between the two groups are equal. Instead, the model can be specified either with the equal variance assumption or with the said assumption relaxed, such that the random variance in the two groups can be examined by comparing the adequacy of the two model-specifications in describing the data using a likelihood-ratio test.

Chapter 2.

Effects of low intensity ambient lighting on refractive development in infant rhesus monkeys (Macaca mulatta)

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2.1 Introduction

Recent studies have consistently found that spending more time outdoors reduces the risk of myopia genesis in children (Dirani *et al.*, 2009; French *et al.*, 2013; Guggenheim *et al.*, 2012; Jones *et al.*, 2012; Rose *et al.*, 2008). Although the exact mechanism remains to be elucidated, the higher lighting intensities that are common in outdoor environments might underlie this protective effect. For instance, elevated laboratory lighting attenuated the reduction of hyperopia that is normally associated with emmetropization (Ashby *et al.*, 2009; Ashby & Schaeffel, 2010; Cohen *et al.*, 2011, 2012; Karouta & Ashby, 2015; Siegwart Jr. *et al.*, 2012). Moreover, rearing animals under elevated lighting reduced the degree of form-deprivation myopia in chicks (Ashby *et al.*, 2009), tree shews (Siegwart Jr. *et al.*, 2012), and rhesus monkeys (Smith III *et al.*, 2012). Finally, elevated lighting reduced the degree of negative-lens-induced myopia in guinea pigs (Li *et al.*, 2014), slowed its development in chicks (Ashby and Schaeffel, 2010) and tree shrews (Siegwart Jr. *et al.*, 2012), although no obvious effects were seen in monkeys (Smith III *et al.*, 2013). These observations provided strong evidence that

elevated lighting levels protect animal eyes against some forms of experimentally induced myopia, supporting the role of brighter outdoor lighting in reducing the risk of myopia in children.

A logical extrapolation of the protective effects of elevated lighting is that low intensity ambient lighting encourages myopia genesis and promotes myopia progression (Norton & Siegwart Jr., 2013). Low intensity ambient lighting has been found to alter the ocular morphology of chickens during postnatal development in a way that resembles myopic ocular changes. The first report of a possible low-light effect on eye growth was from Harrison and McGinnis (Harrison & McGinnis, 1967). They found that rearing chickens under low intensity (0.06~0.09 $\mu W/cm^2*nm$), blue diurnal lighting caused excessive ocular axial elongation, and their eyes became substantially myopic. However, despite some successful replications of their refractive outcome (Bercovitz *et al.*, 1972; Harrison *et al.*, 1968), the role of light intensity remained confounded by the spectral composition of light, until Lauber and Kinnear (Lauber & Kinner, 1979) induced eye enlargement in three different sub-species of chicks using low-intensity white light. This finding suggested that the eye enlargements in the earlier studies were likely lighting intensity, rather than wavelength, associated. It is noteworthy that the eye enlargements observed in these studies did not involve severe corneal flattening and increased intraocular pressure, indicating that the observed phenomena were distinct from constant-light-induced buphthalmia, a glaucomatous condition marked by eye enlargement, high intraocular pressure and severe corneal flattening (Jensen & Matson, 1957; Lauber *et al.*, 1961, 1970). Instead, the low-lighting alterations shared certain biometrical characteristics with other vision-induced experimental models of myopia (specifically, axial elongation due to excessive growth of the vitreous chamber) (for a review see Troilo *et al.*, 2019), suggesting that these two phenomena might have common, if not identical, regulatory mechanisms.

The above pioneering works were conducted before the introduction of practical and precise in vivo biometry measurements and commonly used animal models of myopia (e.g. form-deprivation myopia; Raviola & Wiesel, 1978, 1985), and therefore limited compared with more recent refractive studies. In this regard, Ashby *et al.* (Ashby, Ohlendorf, and Schaeffel, 2009) examined the refractive effects of low intensity lighting using an avian model of form-deprivation myopia. They found that 6-hour daily exposures to low-intensity white lighting (average intensity = 50 lux) for 5 days did not exacerbate form-deprivation myopia in chicks, nor did it make the fellow, untreated eyes more myopic. Similarly, Feldkaemper *et al.* (1999) reported that reducing ambient lighting levels (550 lux) by 2 log units (5.5 lux) for 9 days failed to produce myopia in chickens reared with unrestricted vision (Feldkaemper *et al.*, 1999). Interestingly, these findings were somewhat contrary to those of Cohen *et al.*'s, in which rearing chickens with unrestricted vision in dim light for prolonged treatment periods (90 days) caused reduced corneal power and axial myopia (Cohen *et al.*, 2011;, 2012). Strengthened by the long observation period and periodic biometric measures, the clear distinction between dim- and normal-light emmetropization patterns in the latter study strongly suggested that lower ambient lighting intensity could cause myopia.

Although the studies of Cohen *et al.*'s (2011, 2012) associated low illumination levels with increased risk of myopia, the translational application of the results may be limited due the nature of the ocular component changes (e.g., reduced corneal power) that were associated with the myopic refractive errors. To the best of the authors' knowledge, the effects of low-intensity lighting on refractive development have only been studied in chicks, which possess species-specific ocular anatomical features and light-response mechanisms that might influence refractive development. For example, in contrast to the rod-dominated retinas of humans and non-human primates (cone-to-rod ratio = 1:20), the retinas of chickens are cone-dominated (cone-to-rod ratio = 3:2) (Wisely *et al.*, 2017). These differences may be important because

mice without functional rod pathways do not emmetropize, nor do they develop form-deprivation myopia (Park *et al.*, 2014), which indicates that rods can play a role in vision-dependent refractive development. Considering that the two photoreceptor populations function under different ambient lighting levels, the differences in rod-cone ratio might affect how refractive development proceeds under different ambient lighting levels. Moreover, the chicken cornea is subject to constant-dark- (Troilo & Wallman, 1991) and constant-light-induced corneal flattening (Cohen *et al.*, 2008; Li *et al.*, 1995), the latter of which is a light-intensity-dependent phenomenon (Cohen *et al.*, 2008) that has not been observed in non-human primates (Smith III *et al.*, 2001; Smith III *et al.*, 2003). Finally, rearing animals under quasi-monochromatic lighting appears to affect refractive development in chicks (Foulds *et al.*, 2013; Seidemann & Schaeffel, 2002) and rhesus monkeys (Hung *et al.*, 2018; Smith III *et al.*, 2013, 2015) in a qualitatively different manner, suggesting that ocular mechanisms influenced by ambient lighting in chicks might not be identical to those in primates. In contrast, rhesus monkeys are similar to humans with respect to ocular anatomy, visual physiology (Harwerth & Smith III, 1985), the course of refractive development, and the nature of vision-induced refractive errors (Qiao-Grider *et al.*, 2007; also see the review by Troilo *et al.*, 2019). It is likely that the refractive development of rhesus monkeys reared under low-intensity ambient lighting is etiologically similar to that in humans, making them a promising animal model for the study of dim-light effects on refraction. The purpose of this experiment, therefore, was to examine the effects of low intensity lighting on the refractive development of rhesus monkeys.

2.2 **Methods**

2.2.1 Animal subjects and intervention strategy

Seven infant rhesus monkeys (Macaca mulatta) acquired at 2 weeks of age were the primary subjects. Prior to the onset of the experiment, these monkeys were housed in a primate nursery illuminated by "white" fluorescent lights (GE Ecolux® Starcoat® T8 F32T8/SP35/ECO, General Electric Co., Boston, MA) on a 12-hour light/12-hour dark cycle. The spectral output of the fluorescent lighting was multi-peaked with maximum intensities located at 612 nm and 550 nm (Figure 2-1A). Lighting intensities ranged between 312 – 860 lux as measured in the middle of each caging area with the light sensor facing the ceiling light panel ("normal" light, mean ± standard deviation of ambient lighting intensity = 504 ± 168 lux, correlated color temperature = 3170K).

At 24 ± 2 days of age, monkeys were transferred to another nursery room with reduced diurnal lighting and reared in that room until the end of the dim-light-rearing period (310 ±, 21 days of age). This observation period was longer than that employed in our previous investigations of ambient lighting effects, which ended at approximately 150 days of age (Smith III *et al.*, 2013). We employed an extended observation period for the dim-light monkeys because the myopic refractive changes observed in chickens reared under dim light were slow to develop and appeared to require long treatment periods to become obvious (Cohen *et al.*, 2011).

The experimental lighting was maintained on the same diurnal cycle as the pre-experiment environment. During the light phase, the environment was illuminated by filtering the fluorescent lights through an aluminum-deposited, clear polyester film (Grafix™ Metalized Dura-Lar®, Silver, 0.05mm-thick; Grafix, Maple Heights, Ohio) that was tightly fitted onto the ceiling lighting panels. The resulting room illumination was approximately 55 lux as measured directly under the light panels at waist-level, without significant alterations in

spectral composition (Figure 2-1B). Light levels measured at the front of individual cages with the light meter facing horizontally out of the cage ranged from 7 to 36 lux. Aside from the monkey housing area, the group-socialization area and the connecting area to the housing room were also equipped with fluorescent lights dimmable to the described illumination level. Lighting conditions in these areas remained highly stable throughout the experimental period.

We chose the current ambient lighting level because it facilitates comparisons to recent experiments in chickens (Ashby *et al.*, 2009; Cohen *et al.*, 2011, 2012) and because it should have been sufficient to maintain normal circadian rhythms (Duffy & Czeisler, 2009). Our rearing environment was very dim in comparison to typical outdoor lighting levels. In this respect, our light levels were just above twilight light levels typically encountered outdoors (Koomen *et al.*, 1952). Moreover, our lighting levels were dim in comparison to indoor lighting standards. For example, the lowest maintained illuminance levels recommended by the International Organization for Standardization (ISO 8995-1:, 2002, Lighting of Indoor Work Spaces) for general building areas, educational buildings (student common room), and offices areas for cleric workers are 100, 200, and 500 lux, respectively. In support of our chosen lighting level, a recent intervention trial found that increasing classroom lighting levels from 70 - 100 lux to 440 - 550 lux significantly reduced the magnitude of myopic refractive shifts, slowed axial ocular growth, and lowered the incidence of myopia in children (Hua *et al.*, 2015).

To ensure that the dim-light subjects were not exposed to typical laboratory lighting due to human activity, the following measures were implemented. Animal care personnel who accessed the animals were instructed to dim the light in the connecting area before entering the dim-light monkey housing area. Animals in the dim-light group never left the low-illumination area except for the periodically scheduled measurement sessions. For the measurement sessions, researchers followed the same instructions for accessing the animals and covered the

anesthetized animal's head with light-blocking cloth before transferring them to the lab. During data collection, the lab was illuminated only by projecting a desk lamp onto a wall remote from the animals. Auxiliary lighting was used to facilitate contact lens insertion/removal during optical coherence tomography (OCT) measurements by shining a penlight on the examined eye from the side.

Control data were obtained from age-matched monkeys reared under typical laboratory lighting without visual restriction (normal-light-reared monkeys). Four of these control monkeys were reared during the dim-light experiment, whereas the rest were from previous studies and their data have been published and discussed (Hung, Arumugam, Ostrin, *et al.*, 2018; Hung, Arumugam, She, *et al.*, 2018; Qiao-Grider *et al.*, 2007; Smith III *et al.*, 1999, 2003, 2010, 2013, 2015). The rearing environments for the normal-light monkeys were similar to the pre-experiment environment described above. The general animal husbandry procedures and data collection methods (see below) for these monkeys were identical to those for the dim-light monkeys.

2.2.2 Data collection

Refractive error, corneal power, ocular axial dimensions, and sub-foveal choroidal thickness were measured for both eyes of the dim-light monkeys at the onset of the experiment and periodically throughout the treatment period (every two weeks for the first seven months, then monthly until the end of the experiment). To prepare for data collection, cycloplegia and mydriasis were induced by 1% tropicamide (Akorn Pharmaceuticals, IL, USA) instilled 25 and, 20 minutes before the measurements. Immediately prior to measurement, monkeys were anesthetized with an intramuscular injection of ketamine hydrochloride (15 -, 20 mg/kg) combined with acepromazine (0.15 - 0.20 mg/kg). Topical anesthesia (1% tetracaine ophthalmic solution, Bausch & Lomb Incorporated, Bridgewater, NJ, USA) was applied as

needed. Refractive error was measured using retinoscopy by two experienced examiners and was reported as the mean spherical-equivalent of the spectacle-plane refractive correction for a 14-mm vertex distance. Corneal power in the 3-mm central region was measured along the pupillary axis with a hand-held keratometer (Alcon Auto-keratometer: Alcon, Inc., St. Louis, MO, USA). Three readings were obtained, and the spherical-equivalent corneal powers were averaged. In the case of steep corneas that were outside the measurement range of the keratometry (about 5% occurrence rate among 2-week-old monkeys), a corneal topographer (EyeSys, 2000; EyeSys Vision, Inc. Houston, TX, USA) was used (95% limits of inter-instrument agreement = +0.49 to -0.37 D) (Kee *et al.*, 2002). Anterior chamber depth, lens thickness, vitreous chamber depth, and total axial length were measured using A-scan ultrasonography along the normal to the cornea apex with a 13 MHz transducer (OTI-Scan 1000, Ophthalmic Technologies Inc., Downsview, Ontario, Canada). For the calculation of axial separations between acoustic interfaces, ultrasound velocities in monkey ocular tissue were assumed to be identical to those in the human eye (cornea and lens: 1641 m/s, aqueous and vitreous: 1532 m/s) (Byrne & Green, 2002). Ten separate measurements were taken, and then the calculated dimensions were averaged. The intra-session standard deviations of the A-scan ultrasonography measurements ranged from ± 0.04 to ± 0.08 mm between axial dimension components. Finally, choroidal thickness was measured using spectral-domain optical coherence tomography (SD-OCT; Spectralis, Heidelberg Engineering Inc., Heidelberg, Germany). Specifications for the choroidal thickness analysis have been described previously (Hung *et al.*, 2018). Choroidal thickness data were available from all dim-light monkeys and 7 normal-light monkeys.

All rearing and measurement procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the University of Houston's Institutional Animal Care and Use Committee.

2.2.3 Statistical methods

Statistical analyses were performed using STATA (MP 14; StataCorp, College Station, TX, USA). Mann-Whitney U test and Wilcoxon signed rank test were used respectively for between group and interocular comparisons of refractive error. Paired t-tests were used for the interocular comparisons of ocular parameters. Student's T-tests were employed for the between-group comparisons of ocular parameters and interocular differences in refraction. These cross-sectional analyses were performed for data collected at ages corresponding to the onset of the experiment, at 155 days of age (at the approximate end of the rapid phase of emmetropization in monkeys and the midpoint of the dim-light-rearing period), and at the end of the dim-light exposure period (~310 days of age). Due to differences in the length of follow-up, the availability of normal control data at these two time-points differed (at 155 days of age, n = 41; at ~310 days of age, n = 32). Multi-level, mixed-effect model analyses were used to compare the longitudinal development of refractive error, corneal power, and choroidal thickness of the right eyes between dim-light and normal-light monkeys. Pearson correlation and linear regression were used to characterize the relationship between refractive error and vitreous chamber to corneal radius ratio (VC/CR ratio; both vitreous chamber depth and corneal radius specified in millimeters). The use of VC/CR ratios in these analyses provides a relatively simple, but more sensitive assessment of the contribution of the vitreous chamber to refractive error. In comparison to vitreous chamber depth alone, VC/CR ratios compensate, at least in part, for individual differences in corneal power and their expected effects on refractive error during emmetropization. For statistical inference, the significance level was set to 0.05.

2.3 **Results**

2.3.1 General observations

Dim-light-reared monkeys showed normal, age-related physical development. Body weight gain at ages of 155 ± 6 days were comparable with age-matched, normal-light-reared monkeys of the same birth year (dim-light vs. normal-light, 0.64 kg vs. 0.63 kg, $t = -0.33$, $p = 0.74$). Daily observations did not reveal any abnormal behaviors among the dim-light monkeys. After moving from normal-light housing to dim-light housing, the dim-light monkeys consistently showed higher activity levels during the daily lights-on period and became quiescent during the lights-off period, suggesting that, after transitioning to the dim-light environment, the infant monkeys maintained their light-entrained circadian rhythms that were obtained under the normal intensity, diurnal lighting condition.

Pupil diameters of the dim-light and normal-light monkeys from the same birth year were measured from photographs taken at the fronts of their cages. Only photographs showing the subjects' eyes pointing in a horizontal direction were used (i.e., similar in orientation to the way room illumination was measured at the fronts of individual cages). The diameters in the vertical meridian were reported with the assumption that all pupils were circular. Pupil diameters of the dim-light monkeys observed at approximately 7 weeks of age were 4.98 ± 0.62 mm and 4.96 ± 0.74 mm in the right and left eyes, respectively, and were significantly larger ($t = 7.51$ and 6.22 for right and left eyes, respectively, $p < 0.001$) in comparison to age-matched normal-light-reared monkeys (left eye pupil diameter: 3.58 ± 0.43 mm). During the course of the experiment, the pupils of the dim-light monkeys remained larger than those of the normal-light-reared monkeys (dim light vs. normal light at 25 weeks of age, OD: 5.33 ± 0.37 mm vs. 3.99 ± 0.42 mm, $t = 7.21$, $p < 0.001$; OS: 5.34 ± 0.40 vs. 4.06 ± 0.42 mm, $t = 6.62$, $p < 0.001$). We speculate that the primary effects of larger pupils were to increase retinal illumination and decrease the depth-of-focus (Charman & Whitefoot, 1977; Green *et al.*, 1980).

Using equation (9) from Green, Powers, and Banks (Green *et al.*, 1980) and visual acuity estimates from Boothe, Dobson and Teller (7 cycles/degree for 7-week-old infant monkeys) (Boothe *et al.*, 1985), the depth-of-focus at 7 weeks of age for the dim-light- and normal-light-reared monkeys was 0.20 D and 0.28 D, respectively. Note that the dioptric differences in depth-of-focus between the dim-and normal-light monkeys would likely be smaller after accounting for any reductions in visual acuity due to lower ambient lighting (van Ness & Bouman, 1967). Although the pupil-related changes in depth-of-focus could potentially reduce the efficiency of optical defocus signals, the dioptric differences in estimated depth-of-focus were relatively small. Therefore, it seems unlikely that the differences in pupil size could significantly alter the emmetropization process. It should be noted, however, that because peripheral vision can dominate central refractive development, these calculations may be an oversimplification of the effects of pupil size on emmetropization.

2.3.2 Refractive development

Dim-light monkeys were moderately hyperopic at the onset of the low-ambient-lighting exposure (24 ± 2 days). At this age, their median refractive errors were +3.38 D and +3.50 D in the right and left eyes, respectively ($z = -1.36$, $p = 0.17$). These starting refractive errors were not significantly different from those of age-matched, normal-light-reared monkeys (OD: +3.63 D, $z = 0.46$, $p = 0.65$; OS: + 3.38 D, $z = 0.25$, $p = 0.81$) (Table 2-1).

Whereas the normal-light monkeys exhibited age-related reductions in hyperopia that are associated with emmetropization, the dim-light monkeys showed little evidence of emmetropization. Figure 2-2 illustrates the refractive errors of individual dim-light monkeys as a function of age. The filled and open symbols represent refractive errors for the right and left eyes, respectively. The refractive errors of the right eyes of individual normal-light monkeys are represented by the grey lines in each plot. From these plots, we see that the

refractive development of the dim-light monkeys was highly variable, but there was no evidence for systematic myopic changes. Specifically, monkeys 692 (Figure 2-2A) showed qualitatively normal age-associated reductions in hyperopia in both eyes; the data for this animal remained in the middle of the range of refractions for the normal control monkeys throughout the observation period. On the other hand, the remaining monkeys showed significant deviations from normal-light monkeys in their refractive development. Both monkeys 740 and 734 (Figure 2-2B and C) showed small reductions in hyperopia over the first 6 weeks of treatment, however, both of these monkeys then maintained roughly the same degree of hyperopia for the rest of the treatment period so that at the end of the observation period these monkeys were more hyperopic than the majority of normal monkeys. Interestingly, monkeys 694, 735, 741 and 709 (Figure 2-2, bottom row) exhibited hyperopic shifts for much of the observation period.

Figure 2-3A and B compare the average relative changes in refractive error between dim-light and normal-light monkeys up to 155 days of age. Multi-level, mixed-effect model analysis showed that rearing under dim light increased the variability in refractive change and shifted the mean refractive change trajectory in the less myopic/more hyperopic direction (linear- and quadratic-effect coefficients, OD: $z = 3.24$, $p < 0.01$ and $z = -2.39$, $p = 0.02$; OS: $z = 3.36$, $p < 0.01$ and $z = -2.14$, $p = 0.03$). At the midpoint of the rearing period (155 days), the dim-light monkeys were significantly more hyperopic than age-matched, normal-light-reared monkeys (Figure 2-3C; dim-light vs. normal-light, OD: + 3.13 D vs. + 2.31 D, $z = -2.24$, $p < 0.03$; OS: +3.31D vs. + 2.44 D, $z = -2.49$, $p = 0.01$). Continued exposure to dim light did not change the comparative refractive status between the dim-light and normal-light monkeys, nor did it produce any instances of myopia. At the end of the dim-light exposure period (Figure 2-3D, 310 days), six of the seven dim-light monkeys exhibited refractive errors that were more hyperopic than 95% of the normal-light monkeys (95% confidence interval for the right eyes:

+ 1.56D - + 2.18D, n = 32), and the dim-light monkeys remained significantly more hyperopic than age-matched normal-light monkeys (dim-light vs. normal-light, OD: +2.75 D vs. +1.78 D, $z = -2.78$, $p < 0.01$; OS: + 3.00 D vs. + 1.75 D, $z = -2.93$, $p < 0.01$).

Refractive development in the dim-light monkeys was associated with larger than normal interocular differences (IOD) in refractive error. This characteristic can be seen from the course of refractive development, particularly in monkeys that failed to exhibit an emmetropization-associated reduction of hyperopia. Specifically, for monkeys 734, 694, 735 and 741 (Figure 2-2C - E), obvious interocular difference in refractive errors (≥ 0.5 D) manifested on multiple occasions during the dim-light exposure period. For monkey 709 (Figure 2-2G), binocular hyperopic shifts were accompanied by a concurrent, progressive increase in anisometropia throughout the dim-light exposure period. The anisometropias in the dim-light monkeys frequently fell outside the ±2 SD range of those for the normal-light monkeys over the course of the experiment (Figure 2-4A). The frequency distribution of the magnitude of anisometropia for the dim-light monkeys (Figure 2-4C) was skewed towards larger interocular differences and less leptokurtic than that for the normal-light monkeys (Figure 2-4B), indicating that dim-light monkeys more frequently developed larger anisometropias. At the age of 155 ± 6 days, the mean magnitude of anisometropia was significantly greater in dim-light monkeys than in normal-light monkeys (0.38 ± 0.26 D vs. 0.18 ± 0.18 D, $t = 2.45$, $p = 0.02$). Note that, despite the apparent struggle in maintaining interocular balance, refractive errors in the two eyes of dim-light monkeys remained highly correlated (Pearson correlation, $r = 0.97$, $p < 0.001$).

2.3.3 Corneal power and ocular axial components

Corneal power development appeared unaffected by reduced ambient lighting. At ages corresponding to the onset of the experiment, corneal powers of the dim-light monkeys were well-matched in their two eyes (OD: 61.52 ± 1.15 D, OS: 61.46 ± 1.12 D, $t = 0.28$, $p = 0.79$)

and similar to those of the normal-light monkeys (OD: 61.52 ± 2.03 D, OS: 61.56 ± 1.89 D, t = 0.24, p = 0.80; between-group comparisons, OD: t = 0.01, p = 0.99; OS: t = 0.14, p = 0.89). Figure 2-5A plots the corneal powers of the dim-light and normal-light monkeys as a function of age. As the animals grew, corneal powers in both the dim-light and normal-light monkeys decreased exponentially without any discernable between-group differences in the rate of change (z = 1.08, p = 0.28 and z = -0.56, p = 0.58 for the linear- and quadratic-components, respectively). There were no significant differences in average corneal powers between dim-light and normal-light monkeys at either the midpoint (dim-light vs. normal-light, OD: 55.76 ± 1.76 D vs. 55.80 ± 1.66 D, t = 0.07, p = 0.95; OS: 55.61 ± 1.66 D vs. 55.88 ± 1.72 D, t = 0.40, p = 0.69. Figure 2-5B) or the end of the dim-light exposure period (OD: 54.02 ± 1.52 D vs. 54.10 ± 1.73 D, t = 0.12, p = 0.91; OS: 54.07 ± 1.78 D vs. 54.06 ± 1.76 D, t = -0.01, p = 0.99). These results show that reduced ambient lighting did not exacerbate nor attenuate the age-associated corneal flattening in infant rhesus monkeys.

In both dim-light and normal-light monkeys, age-related changes in refractive error were associated with alterations in ocular axial dimensions. Ocular dimensions measured at the onset of the experiment, the midpoint of the dim-light period (155 days), and the end of the dim-light exposure period (310 days) are compared between dim-light and normal-light monkeys in Table 2-1. In brief, the ocular dimension components were similar in the dim-light and normal-light monkeys at ages corresponding to the onset of the experiment ($p > 0.05$). At 155 (see Table 2-1 and Figure 2-6) and 310 days of age (Table 2-1), the anterior chamber depths and lens thicknesses of the dim-light monkeys were similar to those of age-matched, normal-light-reared monkeys ($p > 0.05$). The average vitreous chamber depth, in contrast, was approximately 0.2 mm shorter in the dim-light monkeys than in the normal-light monkeys at 155 days of age, although this difference was not statistically significant (dim-light vs. normal-light, OD: 9.66 ± 0.50 vs. 9.85 ± 0.31 mm, t = 1.41, p = 0.17; OS: 9.61 ± 0.46 vs. 9.85 ± 0.31 mm, t = 1.82, p

= 0.08) (Figure 2-6). Note that the magnitude of the between-group difference in vitreous chamber depth was optically substantial in the context of monkey eyes, and the relative differences in vitreous chamber depth agreed with the relative differences in refractive status between these two groups (Table 2-1). In addition, refractive errors observed at 155 days were significantly correlated with axial length (r^2 = -0.27, p = 0.04) and vitreous chamber depth (r^2 = -0.43, p < 0.001). These observations suggested that vitreous chamber depth was plausibly the determinant for the variation in refractive errors in dim-light monkeys.

As noted in the methods, a more sensitive assessment on the axial nature of refractive error can be achieved using the VC/CR ratio. For the dim-light monkeys, changes in the VC/CR ratios were highly correlated in changes in vitreous chamber depth (r = 0.77, p < 0.01), suggesting that this metric was a valid representation of axial eye growth. In addition, the changes in VC/CR ratios were inversely correlated with changes in refractive error (r = -0.75, p < 0.001). More importantly, as illustrated in Figure 2-7, at the end of the regular experiment period, there was a strong linear correlation between refractive error and the VC/CR ratio in dim-light monkeys (r = -0.89, p < 0.01); eyes with higher degrees of hyperopia had lower VC/CR ratios, which accounted for 80% of the variance in refractive error (linear regression, r^2 = 0.8, p < 0.01). These data, in association with the corneal power changes described above, indicated that dim-light monkeys' refractive states were mostly determined by ocular axial elongation.

2.3.4 Choroidal thickness changes

Rearing monkeys under dim light caused sustained choroidal thickening in the sub-foveal region. At ages that correspond to the onset of the experiment, sub-foveal choroidal thickness was not significantly different between dim-light and normal-light monkeys (average ± SEM choroidal thicknesses for dim-light vs. normal-light monkeys: OD: 122.76 ± 8.24 μm vs.

121.91 ± 7.30 μm, t = -0.08, p = 0.94, OS: 118.28 ± 9.02 vs. 119.75 ± 6.02, t = 0.14, p = 0.89). Multi-level, mixed-effect model analysis on the right eye data showed that, in both dim- and normal-light monkeys, the choroid underwent age-related thickening (average thickening rate ± SEM = 0.17 ± 0.04 μm/day from the onset of experiment, z = 4.33, p < 0.001). Greater choroidal thickness increases were also associated with more hyperopic refractive errors (3.3 ± 1.5 μm per diopter of relative hyperopia, z = 2.28, p = 0.02). When the influences of age and refractive error to choroidal thicknesses were accounted for, dim-light rearing caused additional relative choroidal thickening in comparison to normal-light rearing, of which the rate was 0.19 μm per day at the onset of the experiment (SEM = ± 0.06, z = 3.11, p = 0.002) and gradually decreased as dim-light rearing continued (z = -2.67, p = 0.01). The resulting choroidal thickness changes are illustrated in Figure 2-8, in which the trajectories of mean choroidal thickness changes of the dim-light and normal-light monkeys became roughly parallel following a rapid departure after the onset of the experiment. At 155 days of age, the changes in choroidal thicknesses were greater in dim-light monkeys than in the age-matched normal-light monkeys (dim-light vs. normal-light: 167.1 ± 6.2 μm vs. 146.9 ± 6.43 μm, t = 2.21, p = 0.04).

2.4 **Discussion**

We examined the effects of low intensity ambient lighting on normal emmetropization and the underlying ocular development in infant rhesus monkeys. We found that exposing infant rhesus monkeys to reduced ambient lighting did not cause myopia; instead, it caused considerable inter-subject and inter-ocular variability in refractive error and reduced the probability that monkeys would emmetropize from the normal, moderate levels of hyperopia found at infancy. Low intensity ambient lighting increased sub-foveal choroidal thickness, but did not cause

systematic alterations in corneal power, anterior chamber depth, or lens thickness. The depth of vitreous chamber remained the primary determinant of refractive error.

2.4.1 Effects of dark-rearing versus dim-light-rearing

Although rearing animals in complete darkness has also been reported to alter refractive development, the alterations produced by constant darkness (dark rearing) and dim-light-rearing strategies are qualitatively different. In chickens, dark rearing greatly increases the between subject variability in refractive error, most commonly resulting in hyperopia. The hyperopic errors in dark-reared chickens are interesting because they are associated with increases in axial length and vitreous chamber depth (Gottlieb *et al.*, 1987; Troilo & Wallman, 1991; Yinon & Koslowe, 1986), which would normally result in relative myopic shifts. The manifest hyperopia in dark-reared chick eyes can be attributed primarily to dramatic corneal flattening (Gottlieb *et al.*, 1987; Troilo & Wallman, 1991; Yinon & Koslowe, 1986). In contrast, as documented above, rearing chickens in dim diurnal ambient lighting consistently produced relative myopic refractive errors that were associated with corneal flattening, but dominated by comparatively much larger increases in vitreous chamber depth (Cohen *et al.*, 2011).

Although there is only a small amount of data available for non-human primates, the most obvious effect of dark rearing in monkeys appears to be an increase in between subject variability in refractive errors. Raviola and Wiesel reported that the refractive errors for the control eyes of two dark-reared, monocularly form-deprived monkeys were +2 D at the end of 9- and 12-month treatment periods (corneal powers appeared to be normal) (Raviola & Wiesel, 1978). As illustrated by the normal control data in Figure 2-2, normal monkeys typically exhibit low degrees of hyperopia at comparable ages. For example, at about 300 days of age, our average normal monkey had a refractive error of +1.87 ± 0.86 D, which suggests that dark rearing does not alter refractive development in monkeys. However, in the only longitudinal

study of dark rearing in monkeys, Guyton *et al.* reported that 2 of 5 dark-reared monkeys developed large degrees of myopia, 2 showed large shifts in the hyperopic direction, and 1 monkey exhibited relatively stable hyperopic refractive errors throughout the treatment period (Guyton *et al.*, 1989). The report did not include ocular parameter measurements, thus the nature of these dark-rearing-induced refractive errors is not known. In contrast to these dark-rearing results, the majority of the dim-light-reared monkeys in this study exhibited relative hyperopic errors that were associated with shorter vitreous chambers; no dim-light monkeys showed relative myopic shifts in refractive error.

The observed differences in the effects of dark rearing and dim-light rearing in both chickens and monkeys are probably not surprising. In particular, the absence of visual input under dark-rearing conceptually precludes emmetropizing responses, a process known to be visually regulated. In this respect, the overall pattern of refractive development in both chickens and monkeys is consistent with unregulated, open-loop behavior. In addition, dark-rearing deprives the eye of the visual signals necessary to maintain a number of ocular circadian rhythms that are important for normal ocular growth and refractive development (for reviews, see Chakraborty *et al.*, 2018; Nickla, 2013).

2.4.2 Inter-species comparisons of the effects of dim light on refractive development

The refractive outcomes in our dim-light monkeys were different from those previously observed in chickens (Lauber and Kinner, 1979; Bercovitz, Harrison, and Leary, 1972; Cohen *et al.*, 2011;, 2012). In the early studies involving chickens, animals reared under very low intensity lighting (~0.12 lux to ~0.34 lux) developed larger equatorial diameters and longer axial lengths, and became myopic relative to those reared under relatively "normal" illumination levels (Bercovitz *et al.*, 1972; Lauber & Kinner, 1979). Note, however, that the "normal" lighting levels in these experiments (Bercovitz *et al.*, ~ 31 lux; Lauber *et al.*, ~ 6.8

lux) approximated the "dim" ambient lighting level in the later studies of Cohen *et al.* (50 lux), in which the association between lower lighting level and more myopia was also observed. Together, these studies showed that the myopiagenic effect of low ambient lighting in chickens was consistent over a relatively large range of the light-intensity continuum. The myopiagenic effects of dim ambient lighting were also qualitatively consistent and robust across subjects. For example, dim-light-reared chickens became relatively less hyperopic than those reared under 500 lux lighting by the, 20^{th} – 30^{th} treatment day and by the 90^{th} treatment day, all but one of 13 dim-light-reared chickens had developed absolute myopia (Cohen *et al.*, 2011;, 2012). For our dim-light-reared monkeys, however, systematic myopic changes were not observed at any point during the experiment. It is unlikely that the failure to observe myopia in our monkeys was related to the length of exposure to dim lighting. We deliberately employed a long treatment period in order to increase the possibility that we would detect any potential myopic shifts. Taking into account interspecies differences in the relative rates of ocular growth (Troilo *et al.*, 2019), the duration of our treatment period substantially exceeded the 90-day dim-light exposure that consistently produced myopia in chickens (Cohen *et al.*, 2011, 2012). Given that the illumination levels in our study were very similar to those in Cohen *et al.*'s, our results indicate that low ambient lighting by itself does not always induce myopia, at least not in rhesus monkeys.

2.4.3 Effects of dim, elevated and typical laboratory lighting on emmetropization in monkeys.

Based primarily on the results in chickens, it has been proposed that ambient light intensity quantitatively changes the course and/or endpoint of emmetropization with dim and elevated lighting promoting myopia and hyperopia, respectively (Norton & Siegwart Jr., 2013). However, that may not be case in monkeys. We previously showed that elevated ambient

lighting did not alter emmetropization in monkeys reared with unrestricted vision, nor did it alter the time course or the degree of compensation to imposed hyperopic defocus in treated eyes or the course of emmetropization of the fellow, untreated eyes, i.e., monkeys reared under elevated ambient lighting emmetropized normally (Smith III *et al.*, 2013). On the other hand, the present study showed that monkeys reared with unrestricted vision under dim light largely failed to emmetropize. The absence of consistent emmetropizing responses in dim-light-reared monkeys suggests that there might be an ambient lighting intensity threshold for the initiation and regulation of emmetropization. Supra-threshold ambient lighting (i.e., typical laboratory lighting and elevated lighting) seems to ensure a high probability of normal emmetropization, speculatively by allowing accurate encoding and/or proper amplification of signals. In contrast, sub-threshold lighting levels may reduce the accuracy in signal encoding and/or the gain in signal processing, leading to a dampened emmetropization process. A possible consequence of such alterations is that hyperopic and myopic defocus, which are thought to regulate refractive development (Hung, Crawford, & Smith III, 1995; Schaeffel, Glasser, & Howland, 1988; for a review see Troilo *et al.*, 2019), must be optically stronger (higher in nominal power) in order to produce functionally adequate "go" and "stop" signals under dim light. In this respect, it is possible that the larger-than-normal fluctuations in anisometropia observed in the dim-light monkeys reflected an increase in the threshold defocus level required to "trigger" mechanisms that are responsible for maintaining isometropia or overcoming anisometropia.

2.4.4 The effects of dim light on corneal power

Reduced ambient lighting had little effect on corneal power in rhesus monkeys. The degree and time course of the age-related reduction in corneal power were similar in monkeys reared under reduced, normal (Qiao-Grider *et al.*, 2007), and elevated ambient lighting levels (Smith III *et al.*, 2013). Such stability is important because a relatively small amount of defocus is sufficient

to consistently induce compensatory refractive changes in rhesus monkeys (Hung, Crawford, & Smith III, 1995). On the other hand, in the studies that reported dim-light-associated myopic shifts in chickens, the myopic shifts appeared to be associated with corneal flattening (Bercovitz, Harrison, and Leary, 1972; Cohen *et al.*, 2011; Harrison, Bercovitz, and Leary, 1968; Harrison and McGinnis, 1967). Specifically, in the study of Cohen *et al.*, substantial differences in corneal power (2.2D flatter) were observed between chickens reared under normal- and dim-light on as early as the, 20^{th} day of exposure and clearly preceded any meaningful differences in axial length (Cohen *et al.*,, 2011). Following the reasoning in section 2.4.3, these early reductions of corneal power might have augmented the reduced regulatory signals by increasing the magnitude of hyperopic defocus, thereby triggering the emmetropization process that might otherwise be quiescent at low lighting levels. It is possible that these animals subsequently developed myopia because once axial elongation was triggered, the resulting low levels of myopic defocus were not sufficient to stop axial elongation. If this is correct, we would predict that optically imposed defocus might similarly result in higher than expected degrees of myopia in infant monkeys.

2.4.5 Possible role of dopamine in light-intensity-induced refractive changes

Animal studies suggest that the protective effects of elevated lighting against myopia in children is associated with retinal dopamine (Rose *et al.*, 2008). It has been established that the synthesis, release, and turnover of retinal dopamine can be induced by ambient light (Iuvone *et al.*, 1978) in an intensity-dependent manner (Brainard & Morgan, 1987; Proll *et al.*, 1982). In chicks and rhesus monkeys, elevated laboratory lighting inhibits form-deprivation myopia (Ashby and Schaeffel, 2010; Smith III, Hung, and Huang, 2012). The role of dopamine in this process was evident in that intravitreal injection of spiperone, a D_2 receptor antagonist, abolished these protective effects in chicks (Ashby and Schaeffel, 2010). Cohen *et al.* further

demonstrated that, in chicks, both refractive development and the production of vitreal 3, 4-dihydroxyphenylacetic acid (DOPAC, the primary metabolite of dopamine) were light-intensity dependent. Specifically, higher ambient-light intensities were associated with less myopia and higher dopamine levels (Cohen *et al.*, 2012). Largely based on this observation, Norton and Siegwart (Norton & Siegwart Jr., 2013) proposed a working model, in which light-intensity dependent changes in retinal dopaminergic activity allows ambient light level to quantitatively alter the end point for refractive development.

There are, however, challenges to this theory. For example, Stone *et al.* did not find correlations between dopamine/DOPAC, outdoor rearing, and the transient inhibitory effects of outdoor rearing on form-deprivation myopia (Stone *et al.*, 2016). With respect to the light intensity dependency of retinal dopamine, a recent study by Landis *et al.* (Landis *et al.* IOVS, 2019;60:ARVO E-Abstract 3152) showed that, although retinal DOPAC production in mice was dependent on ambient light intensity, under long-term exposure to altered ambient illumination levels (0.005 lux, 50 lux, and 15,000 lux during the light phase of the diurnal lighting cycle), retinal dopamine levels remained similar across the different lighting levels. These findings suggested that, under chronic exposures to different ambient illuminations, adaptive mechanisms might respond and maintain a relatively constant retinal dopamine level, thus retinal dopamine availability might not always be light-intensity dependent. With respect to the potential light dependency of refractive development, we found in this and in a previous study (Smith III *et al.*, 2013) a lack of apparent correlation between ambient lighting intensity and the degree of refractive error in rhesus monkeys reared without visual restrictions. Both lines of evidence suggest that the effects of lighting level on the degree of refractive error may not be explained by the light-intensity dependency of a single molecule. It is possible that retinal dopamine is part of a more complex molecular mechanism that mediates the lighting-intensity effect on refractive development.

2.4.6 Dim-light-induced choroidal thickness changes

To the best of the authors' knowledge, this is the first report of sustained, dim-light-induced choroidal thickening in non-human primates. In the early works of Harrison and McGinnis (Harrison & McGinnis, 1967), choroidal thickness increased dramatically (3-4 folds) in low intensity blue-light-reared chickens, although the results might be confounded by the narrow spectral compositions of the ambient lighting. In this respect, a subsequent study conducted by the same group not only failed to replicate these choroidal changes, but rather found thinner choroids in comparison to the normal-light-reared chickens (Harrison *et al.*, 1968). More recently, Lan *et al.* found that the chicken choroid slightly thinned after moving from normal (500 lux) to bright ambient lighting (15,000 lux). When these chickens were subsequently removed from bright light, placed under normal lighting for 2 hours, and then exposed to darkness for another 2 hours, their choroidal thicknesses increased (Lan *et al.*, 2013). In a somewhat analogous manner, in humans, nightly exposure to 1,000 lux lighting instead of 150 lux lighting was reported to induce thinning of the sub-foveal choroid (Ahn *et al.*, 2017), whereas dark adaptation was reported to increase sub-foveal choroidal thickness (Alagöz *et al.*, 2016). Despite the substantial methodological differences, these studies suggest a link between relative choroidal thickening and lower ambient lighting levels.

Did dim-light-associated choroidal thickening influence refractive development? In many species, the choroid thickens in response to myopic defocus and thins in response to hyperopic defocus (Nickla and Wallman, 2010). At least in chicks, the magnitude of defocus-induced choroidal thickness change was sufficiently large to serve as an intermediate-acting mechanism for reducing refractive error (Wallman *et al.*, 1995; Wildsoet and Wallman, 1995). For our dim-light monkeys, however, it is unlikely that the relative increases in choroidal thickness were induced by defocus, as the animals were hyperopic throughout the experiment. It is also

unlikely that the direct optical effects induced by relative choroidal thickening constituted a significant dioptric force for refractive regulation. Although the choroid of the dim-light monkeys rapidly thickened relative to those of the normal-light monkeys, the mean difference in thickness observed at 155 days of age (~20 μm) remained small and was not sufficient to substantially alter the eye's effective refractive state. In fact, even if such a difference took place in 24-day-old monkeys (in which the increase in choroidal thickness would effectively augment the natural hyperopia) (Qiao-Grider *et al.*, 2007), the resulting relative hyperopia (< + 0.25 D) associated with the observed degree of choroidal thickening would appear to be negligible considering the level and between-subject variability in refractive error that is naturally present early in life (in our experiment, the mean ± standard deviation of refractive error at baseline = +3.82 ± 1.83 D). Therefore, the dim-light-associated relative choroidal thickening did not appear to contribute significantly to the dioptric changes observed in the dim-light monkeys. Although the changes in choroidal thickness appeared to be predictive of the hyperopic shifts observed over the course of the treatment period in some animals, the underlying mechanisms and implications remain to be investigated.

2.4.7 Clinical implications, limitations, and future directions

Although low ambient lighting is not always myopiagenic for primates, it does appear to compromise normal emmetropization in rhesus monkeys. From a refractive error management standpoint, if a light-intensity threshold for emmetropization exists, determining this level has important implications. For rhesus monkeys, this critical level appears to be within a narrow range between about 50 and 500 lux, (approximately the average "dim" and "normal" lighting levels in our laboratory), which is somewhat alarming because humans can encounter comparable low light levels indoors (Ostrin, 2017). If human refractive development responds

to dim ambient lighting in the same way as rhesus monkeys, reduced indoor lighting may indeed be a risk factor of abnormal refractive development.

A limitation of our study was that the illumination level, as well as the duration of exposure, was not representative of real-world scenarios. In addition, whereas transitioning between relatively lower and higher ambient lighting frequently takes place in daily life; our subjects were deprived of such opportunities. Transitioning between ambient lighting conditions might be physiologically impactful because temporal contrast might serve as an additional trigger for retinal dopamine release. These limitations suggest that the observed refractive effects of dim lighting might be largely exaggerated. Nonetheless, our study highlighted the importance of proper indoor lighting, especially for young children who are in the early stages of emmetropization.

From our findings, it is not clear from a mechanistic perspective how dim ambient lighting influences refractive development. Specifically, our data did not show whether the ocular regulatory mechanisms were not detecting defocus signals or that these mechanisms were simply not responding to appropriately encoded signals. This issue has practical significance because current optical interventions for myopia are either based on or thought to be related to the response of regulatory mechanisms to imposed defocus signals (for a review see Troilo *et al.*, 2019). For example, if dim ambient lighting causes growing eyes to misinterpret the nominal sign or magnitude of optical regulatory signals, one would expect that dim lighting has the potential to render optical treatment strategies ineffective. In this respect, it will be important to investigate the effects of dim ambient lighting on the various forms of experimentally induced refractive errors in non-human primates.

Acknowledgement

The authors thank Diana Tran for her contribution in research data management. This work was supported by National Institutes of Health Grants EY-03611 and EY-07551, funds from the Brien Holden Vision Institute, and the University of Houston Foundation.

Tables and Figures

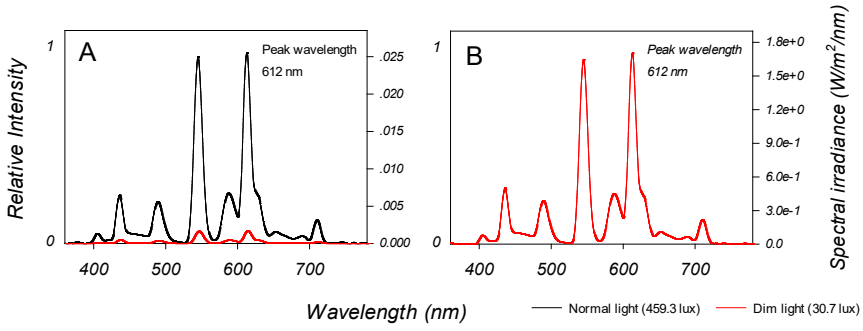
Table 2-1. Refractive errors and ocular parameters

	Baseline				Midpoint of the dim-light-rearing period				End of the dim-light-rearing period			
	Dim Light (24 ± 2 days, n = 7)		Normal Light (24 ± 4 days, n = 41)		Dim Light (155 ± 6 days, n = 7)		Normal Light (148 ± 10 days, n = 41)		Dim Light (310 ±, 21 days, n = 7)		Normal Light (306 ± 10 days, n = 32)	
	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
Median RE (D)	+ 3.38	+ 3.5	+ 3.63	+ 3.38	+3.13*	+ 3.31*	+ 2.31*	+ 2.44*	+ 2.75*	+ 3.00*	+1.78*	+1.75*
Corneal power (D)**	61.52 ± 1.15	61.46 ± 1.12	61.52 ± 2.03	61.56 ± 1.89	55.76 ± 1.76	55.61 ± 1.66	55.80 ± 1.66	55.88 ± 1.72	54.02 ± 1.52	54.07 ± 1.78	54.10 ± 1.76	54.05 ±1.78
Anterior chamber depth (mm) **	2.54 ±0.13	2.54 ± 0.11	2.65 ± 0.29	2.64 ± 0.30	3.11 ± 0.11	3.08 ± 0.09	3.06 ± 0.28	3.09 ± 0.29	3.21 ± 0.12	3.18 ± 0.14	3.17 ± 0.22	3.14 ± 0.26
Lens Thickness (mm) **	3.73 ± 0.10	3.72 ± 0.11	3.50 ± 0.29	3.52 ± 0.00	3.66 ± 0.13	3.67 ± 0.10	3.63 ± 0.21	3.61 ± 0.21	3.67 ± 0.13	3.70 ± 0.17	3.60 ± 0.12	3.62 ± 0.13
Vitreous chamber depth (mm) **	8.51 ± 0.34	8.5 ± 0.32	8.64 ± 0.31	8.64 ± 0.31	9.66 ± 0.50	9.61 ± 0.46	9.85 ± 0.31	9.85 ± 0.31	10.22 ± 0.63	10.25 ± 0.59	10.53 ± 0.37	10.55 ± 0.35

*: Significant between-group difference.
** Mean ± SD of mean.

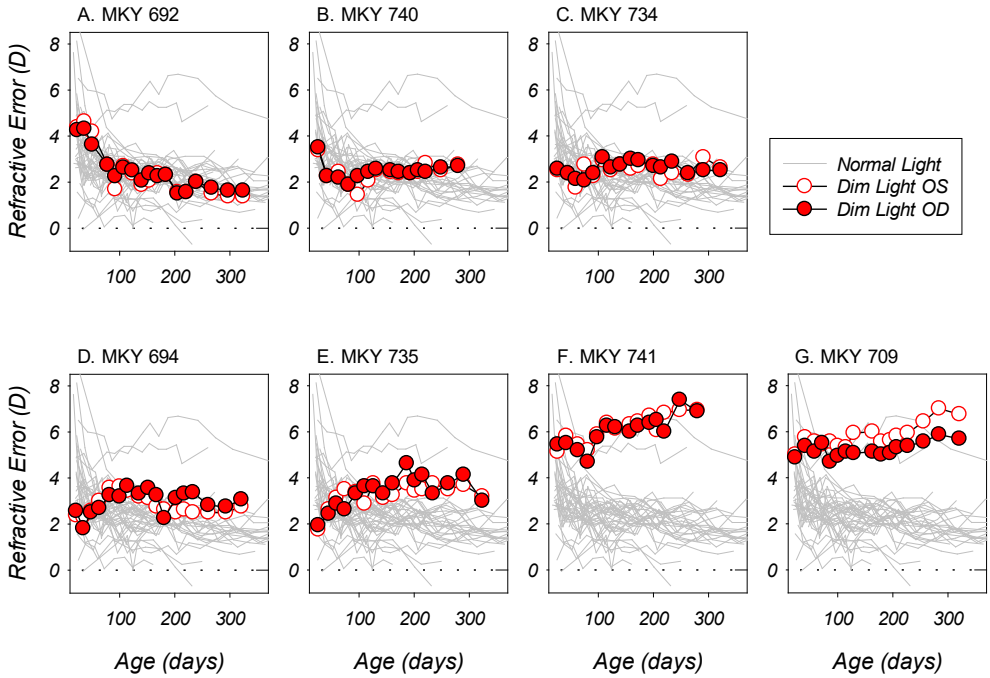
Refractive errors, corneal powers, and ocular axial dimensions of monkeys reared under dim light and normal light at baseline, 155 days of age and 310 days of age. The asterisks indicated significant between-group differences.

Figure 2-1: Spectral irradiance of dim light



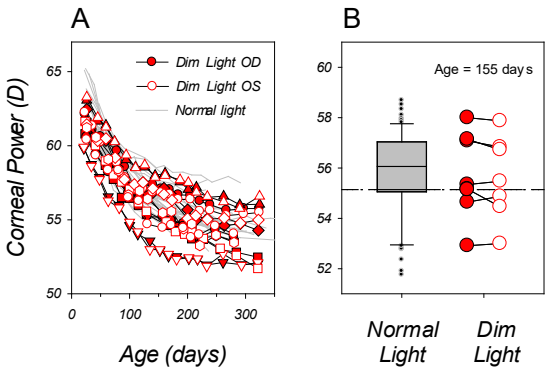
The spectral irradiance (Spectrophotometer CL-500A, Konica Minolta Sensing America, Inc. Ramsey, NJ, USA) of two representative measurements for the normal (corresponding illuminance level: 459.31 lux) and dim ambient lighting levels (corresponding illuminance level: 30.7 lux). **Panel A**. Spectral irradiance for normal lighting (black line) compared to that for dim lighting (red line). **Panel B**. Spectral irradiance for the dim lighting condition plotted on an expanded y-axis to illustrate the similarities in the spectral composition of the light under the dim and normal ambient lighting conditions.

Figure 2-2: Refractive development for individual dim light monkeys reared without visual restriction



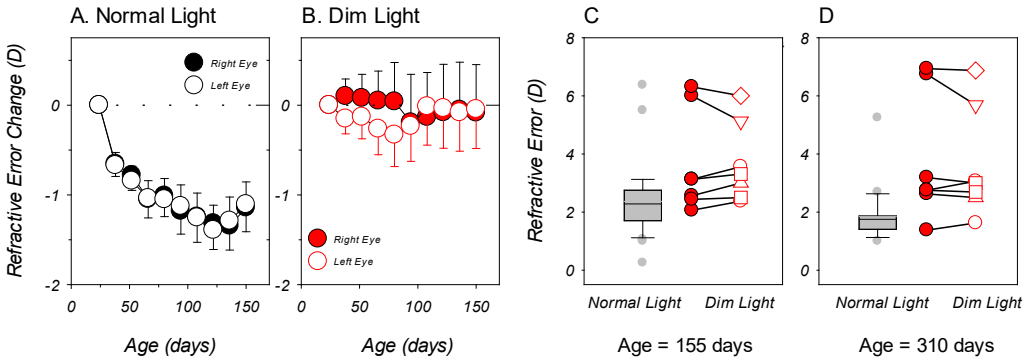
Refractive errors of individual dim-light monkeys plotted as a function of age. Filled and open symbols represent the refractive errors in the right and left eyes, respectively. Refractive errors for the right eyes of normal-light monkeys (n = 41) are plotted in the same manner as thin, solid lines. Only one dim-light monkeys showed age-related reductions in hyperopia that were consistently near the middle of the normal range (panel A). Other monkeys either maintained the same degree of hyperopia (panels B and C) or developed progressive hyperopic shifts in refractive error (second row).

Figure 2-3: Corneal power developments



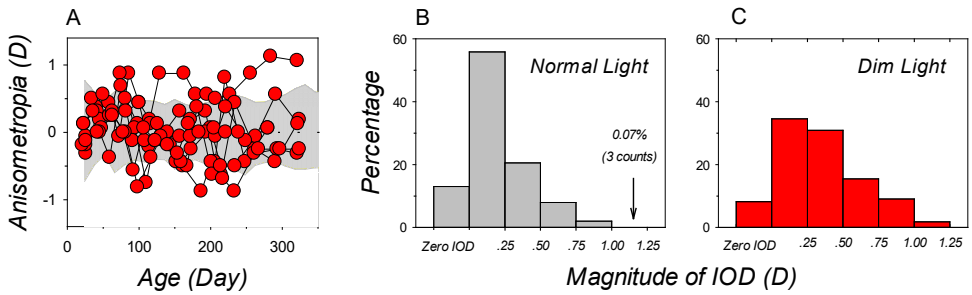
A. Corneal powers for the right (filled symbols) and left eyes (open symbols) of the dim-light monkeys plotted as a function of age, along with the corneal powers of the right eyes of normal control monkeys (represented by the thin solid lines). With one exception, all dim-light monkeys' corneal power development fitted well into the pattern observed in normal-light monkeys. B. Corneal powers of the dim-light monkeys at 155 days of age compared with those of age-matched, normal-light monkeys. Symbols above and below the error bars represent the outliers. The horizontal line inside the boxplot indicates the median corneal power, whereas the horizontal dashed line across the panel represents the mean corneal power of normal monkeys. There was no statistically significant difference in corneal power between the two groups at 155 days of age.

Figure 2-4: Mean refractive change and refractive error at the end of the experiment



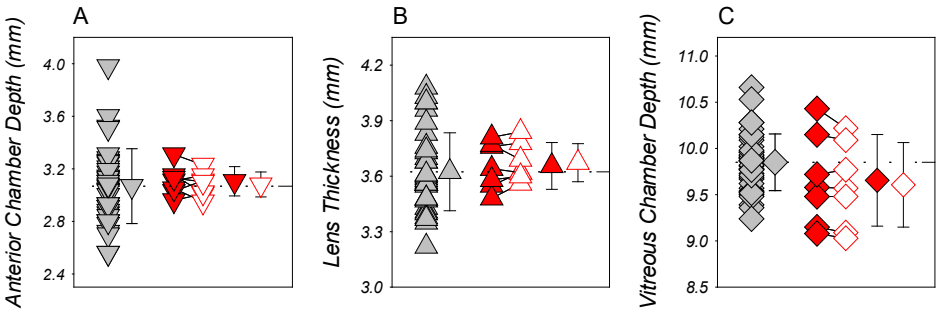
Panels A and B: Mean refractive-error changes relative to baseline values plotted as a function of age for the right (filled symbols) and left eyes (open symbol) of the normal-light and dim-light monkeys, respectively. Dashed lines represent zero change in refractive error. Error bars represent ±1 standard deviation from the mean refractive-error change. Normal-light monkeys (panel A) exhibited age-related myopic shifts in both eyes, whereas dim-light monkeys developed highly variable refractive changes that averaged between 0 ~ -0.5D throughout the experiment (panel B). In comparison to the age-matched normal-light monkeys, these changes resulted in more hyperopic refractive states in the dim-light monkeys at both ~155 days (Panel C) and ~310 days of their age (Panel D).

Figure 2-5. Interocular difference in refractive error



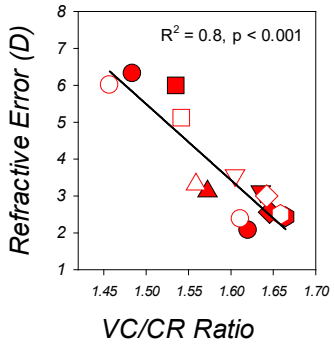
Panel A: Interocular differences (IOD) in refractive error of individual dim-light monkeys plotted as function of age. The grey area represents the ± 2 standard deviation range of IODs for the normal-light monkeys. Panels B and C: Frequency distributions for the magnitude of IODs in refractive error for the normal-light and dim-light monkeys, respectively. The first columns in these panels represent the percentage of samples that had zero interocular difference.

Figure 2-6. Ocular axial dimensions at the end of the experiment



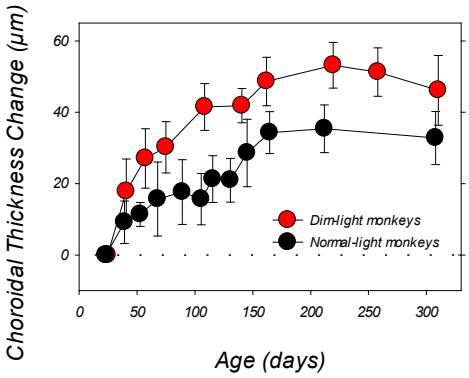
Ocular axial parameters for the dim-light and normal-light monkeys at 155 days of age. Data from the right (red, filled symbol) and left eyes (red, open symbol) of the dim-light monkeys are plotted on the right side of each panel. Data from the right eyes of individual normal-light monkeys (grey filled symbols) are plotted on the left in each panel. The symbols with error bars to the right of the individual data represent the mean and ±1 standard deviation from the mean for the corresponding ocular parameter. There were no statistically significant differences in ocular axial parameters between the dim-light and normal-light monkeys.

Figure 2-7. Correlation between VC/CR ratio and refractive error



Refractive error for the right (filled symbols) and left eyes (open symbols) of the dim-light monkeys at 155 days of age plotted as a function of the vitreous chamber depth to corneal radius ratio (VC/CR ratio). Both vitreous chamber depth and corneal radius in the calculation of the VC/CR ratio are specified in millimeters. The two eyes from the same monkey are represented by the same shaped symbol.

Figure 2-8. Choroidal thickness development



Changes in sub-foveal choroidal thickness relative to the onset of the experiment for dim-light (red symbols) and normal-light (black symbols) monkeys plotted as functions of age. For a given animal the data for the right and left eyes were averaged. The symbols in the figure represent group averages; the error bars represent ±1 standard error of the mean. Compared with normal-light monkeys, dim-light monkeys showed sustained choroidal thickening in the sub-foveal region.

Chapter 3.

The development of and recovery from form-deprivation myopia in infant rhesus monkeys reared under reduced ambient lighting

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3.1 Introduction

Myopia is a multifactorial refractive disorder (Flitcroft, 2013; Wallman & Winawer, 2004), of which the prevalence typically starts to increase during childhood (for a review, see: Holden *et al.*, 2016). Because the underlying ocular changes are irreversible, identifying the environmental factors that associate with the genesis and development of myopia has significant implications for reducing the growing myopia-associated public health burden. Epidemiological studies have shown that children who spend more time outdoors have higher hyperopic refractive errors and a lower chance of developing myopia (Dirani *et al.*, 2009; French, Ashby, Morgan, & Rose, 2013; Guggenheim *et al.*, 2012; Jones *et al.*, 2012; Rose *et al.*, 2008; Wu *et al.*, 2013), speculatively attributed to the higher luminance levels typically associated with outdoor environments. In support of this view, elevated ambient lighting has been shown to slow the development of form-deprivation myopia (FDM) in tree shrews (Siegwart Jr. *et al.*, 2012) and reduce its magnitude in chicks and rhesus monkeys (Ashby *et al.*, 2009; Smith III *et al.*, 2012). These effects on FDM appear to be light-intensity dependent

(Karouta and Ashby, 2015). In addition, emmetropization-associated reductions in hyperopia can be slowed or reduced by elevated ambient lighting in chicks and tree shrews (Ashby *et al.*, 2009; Ashby & Schaeffel, 2010; Cohen *et al.*, 2011, 2012; Siegwart Jr. *et al.*, 2012), although not in rhesus monkeys (Smith III *et al.*, 2013). These findings indicate that higher lighting levels may be protective against myopia.

The positive findings with elevated lighting have motivated further investigations as to whether lower lighting levels might contribute to human myopia genesis. However, the limited studies on the effects of reduced ambient lighting on emmetropization showed substantial inter-species discrepancies. Cohen *et al.* found that, in comparison to animals reared under typical laboratory lighting (500 lux), chickens reared under dim lighting (50 lux) developed absolute myopia (Cohen *et al.*, 2011, 2012). Their findings, along with the early works of Bercovitz *et al.* (Bercovitz *et al.*, 1972) and Lauber and Kinner (Lauber & Kinner, 1979), suggested that dim light could be myopiagenic. On the other hand, rearing infant rhesus monkeys under reduced ambient lighting interfered with normal emmetropization, increased both inter-individual and interocular variability in refractive errors, but it did not cause myopia (She *et al.*, 2020). These observations suggested that, for primates, dim light does not always cause myopia in otherwise emmetropizing eyes, but could compromise the visual regulation that governs normal refractive development.

For most laboratory animals, emmetropization does not typically end with spontaneous myopia (Cohen *et al.*, 2011; Graham and Judge, 1999; Norton and McBrien, 1992; Qiao-Grider *et al.*, 2007; Wallman, Adams, and Trachtman, 1981; Zhou *et al.*, 2006). However, myopia that is qualitatively similar in nature to common myopic errors in children can be induced by specific visual manipulations in many species (Barathi *et al.*, 2008; Howlett and McFadden, 2006;, 2009; Hung, Crawford, and Smith III, 1995; Raviola and Wiesel, 1985; Smith III and

Hung, 2000; Troilo and Judge, 1993; Troilo and Wallman, 1987; Wallman, Turkel, and Trachtman, 1978; McBrien, Cornell, and Gentle, 2001; Siegwart Jr. and Norton, 1998). In this regard, a related query is whether dim light could alter the development of these visually induced myopias. Direct assessment of this issue was first carried out by Ashby *et al.* (Ashby *et al.*, 2009) using an avian form-deprivation model of myopia. In their experiment, form-deprived chicks were placed under dim light (50 lux) for 6 of the 12 hours of the daily lights-on cycle from 1 to 4 days of age. In comparison to normal ambient lighting (consistently 500 lux throughout the daily lights-on hours), this intermittent dim-light exposure did not cause more form-deprivation myopia, nor did it produce greater-than-normal reductions in hyperopia in their contralateral, untreated eyes. These observations suggested that, for chickens, dim light might not be a risk factor for vision-induced myopias.

To date, the effects of low ambient lighting levels on visually induced myopias has only been studied in chickens. Given the limited information and the implications for understanding human myopias, we investigated the development of monocular FDM under reduced ambient lighting in non-human primates. In addition, we also examined the recovery from FDM under dim light. Recovery from FDM, i.e. the reduction in the diffuser-induced myopic anisometropias, has been observed in many species (Howlett & McFadden, 2006; Qiao-Grider *et al.*, 2004; Siegwart & Norton, 1998; Wallman & Adams, 1987) and is regarded as the one of the first clear indicators of visual regulation of refractive development (Qiao-Grider *et al.*, 2004; Schaeffel & Howland, 1991; Troilo *et al.*, 2019; Wildsoet & Schmid, 2000). To the best of the authors' knowledge, there were no prior longitudinal evaluations on the effects of lower ambient lighting levels on the recovery from FDM. The study of this phenomenon could provide information on whether and how the visual regulatory mechanisms for refractive development are influenced by low ambient lighting levels.

3.2 **Method**

3.2.1 Primary subjects and pre-dim-light rearing conditions

Seven infant rhesus monkeys (Macaca mulatta) were acquired at two weeks of age, and then reared in a climate-controlled housing environment until the onset of the experiment. This housing area was illuminated by broadband, white fluorescent lights (GE Ecolux® Starcoat® T8 F32T8/SP35/ECO, General Electric Co., Boston, MA) on a 12 hour-light/12 hour-dark diurnal cycle. The lighting intensity during the daily light phase (7AM – 7PM), as measured at waist-level (approximately the height of the junction between the upper and lower cages), ranged between 312 – 860 lux ("normal" lighting, average illuminance = 504 ± 168 lux, correlated color temperature = 3170K) (She *et al.*, 2020).

3.2.2 Experimental strategies

Starting at 25 ± 3 days of age, the subjects were reared under reduced ambient illumination with concurrent monocular form deprivation until 154 ± 7 days of age (dim-light-diffuser period). At that time the diffuser lenses were removed, these dim-light-reared, form-deprived monkeys (DL-FD monkeys) then experienced unrestricted vision in both eyes in the dim ambient lighting until 337 ± 10 days of age (dim-light-recovery period).

3.2.2.1 Experimental "dim" lighting

The reduced ambient lighting (dim light) in the current experiment was maintained throughout the light phase of the diurnal lighting cycle (7AM – 7PM). It was produced by filtering the fluorescent lighting through an aluminum deposited, semi-reflective film (Grafix™ Metalized Dura-Lar®, Silver, 0.05mm-thick; Grafix, Maple Heights, Ohio) that was closely fitted to the ceiling light panels. Ambient illumination was reduced to 55 ± 9 lux at waist level without

significant alterations in the spectral composition of the ambient lighting. Light measures obtained with the meter directed out of the front of individual cages, which reflects the visual environment that the animals commonly encountered, varied from 7 to 36 lux (average intensity = 15 ± 8 lux) (She *et al.*, 2020). This average ambient lighting level, which was chosen in part to facilitate between-study comparisons (Cohen *et al.*, 2011 and, 2012, Ashby *et al.*, 2009), was identical to that employed in our previous investigation (She *et al.*, 2020). A more detailed rationale for the chosen lighting level can be found in She *et al.* (2020).

3.2.2.2 Form-deprivation

Monocular form deprivation was produced using "light perception" (LP) Bangerter occlusion foils (Fresnel Prism and Lens Co., Bloomington MN, USA) that were attached to zero-power (i.e., plano) carrier lenses (diffuser lenses). At the onset of the dim-light-diffuser period, each of the DL-FD monkeys was fitted with a light-weight helmet that held the diffuser lens in front of the treated, form-deprived eye and a plano lens in front of the fellow control eye. The goggle-like helmets were worn continuously except for the brief daily periods when the lenses were cleaned. Throughout the daily light-on cycle, the helmets were inspected frequently and adjusted as needed to ensure proper fit and optics.

The diffuser lenses reduced spatial contrast without significant alterations in the effective lighting levels. Specifically, LP Bangerter diffusers dramatically reduced the modulation transfer at low- and mid-range spatial frequencies and virtually eliminated contrast for higher spatial frequencies (Pérez *et al.*, 2010; Smith III & Hung, 2000). For the specific diffusers employed in the current experiment, viewing through the diffuser lenses was found to reduce human contrast sensitivity by 1.25 log units at 0.1 cycle/degree (cpd) and by 2 log units at 0.5 cpd, with higher spatial frequencies being undetectable (Smith III & Hung, 2000). On the other hand, the decrease in light transmission through the diffusers was very small. As shown in

Figure 3-1, ambient- and diffuser-attenuated-illumination levels had a linear relationship in log-log coordinates in the dim-to-normal range of ambient illuminations. This relationship predicts an average reduction in ambient illumination on the order of 0.04 log units, which appeared negligible both in comparison to the light-level difference between the two experimental paradigms (approximately 1 log unit) and to the operating range of the primate eye.

3.2.3 Control data

The primary control subjects were a group of age-matched, monocularly form-deprived monkeys previously reared with the same LP Bangerter diffusers under our "normal" laboratory lighting levels (normal-light-reared, form-deprived, or NL-FD monkeys, n = 16). The refractive data for these subjects have been published and discussed (Hung *et al.*, 2018; Smith III & Hung, 2000; Smith III *et al.*, 2012; Smith III *et al.*, 2002). Data from monkeys previously reared with unrestricted vision under "normal" laboratory lighting (normal controls, n = 41) were included as a reference for the vision-induced changes in refractive development (Hung *et al.*, 2018; Hung *et al.*, 2018; Qiao-Grider *et al.*, 2007; Smith III *et al.*, 1999, 2003, 2010, 2015). In addition, refractive error and choroidal thickness data obtained from monkeys that were previously reared with unrestricted vision under identical dim-light conditions (dim-light or DL-controls, n = 7) (She *et al.*, 2020) were included in some analyses (see section 3.2.4). Finally, the recovery data from monkeys reared under normal lighting levels with either different strength monocular diffuser lenses or monocular LP diffusers combined with some brief periods of unrestricted vision each day (n = 13) (Smith III *et al.*, 2002; Smith III & Hung, 2000) were included to help characterize the recovery from FDM under typical ambient lighting. The husbandry strategies, diffuser-treatment paradigm, and data collection methodologies for all control subjects were identical to those for the DL-FD monkeys.

3.2.4 Outcome measures and data collection

Refractive errors, ocular axial dimensions, corneal powers, and choroidal thicknesses were measured periodically. Before each measurement session, the animals were cyclopleged (1% tropicamide instilled 25 and, 20 minutes before the measurements) and anesthetized (intramuscular injection of 15 -, 20 mg/kg of ketamine hydrochloride, combined with 0.15 - 0.20 mg/kg of acepromazine). During the measurement sessions, supplemental topical anesthesia was applied as needed (1% tetracaine ophthalmic solution). Procedures were implemented to ensure that the animals were not exposed to higher ambient lighting during the data collection activities (She *et al.*, 2020).

Refractive error was determined using retinoscopy by two experienced examiners and reported as the mean spherical-equivalent of the spectacle-plane refractive correction for a vertex distance of 14 mm. In previous studies of FDM, interocular differences (IOD, form-deprived eye refraction - control eye refraction) in refractive errors were used as the primary outcome-measure because, with a monocular treatment paradigm, refractive development in the two eyes is largely independent: the lens- or diffuser-treated eyes develop ametropias corresponding to the nature of treatment, whereas their contralateral, untreated eyes typically emmetropize to a relatively normal level (Hung, Arumugam, She, *et al.*, 2018). In this instance, the refractive stability in the untreated eye allows the use of IODs as a sensitive measure of any treatment effects. This metric might not be optimal for the current experiment because our previous study of emmetropization under dim ambient lighting suggested that control eye refractive development could be altered by the dim-light paradigm (She *et al.*, 2020). Therefore, in this study, we used the absolute refractive error in addition to the IODs in refractive error to determine the main effects of dim-ambient lighting.

Corneal powers and ocular axial dimensions were measured to examine the nature of refractive errors. Corneal power was determined using either a hand-held keratometer (Alcon Auto-keratometer: Alcon, Inc., St. Louis, MO, USA) or, in the case when the corneas were too steep (> 62 D, about 5% occurrence rate among 3-week-old monkeys), a corneal topographer (EyeSys, 2000; EyeSys Vision, Inc. Houston, TX, USA) (95% limits of inter-instrument agreement = +0.49 to -0.37 D) (Kee *et al.*, 2002). The spherical-equivalent of three independent measurements were averaged to represent the corneal power along the pupillary axis in the 3-mm central region of the cornea. Anterior chamber depth, lens thickness, vitreous chamber depth, and total axial length were assessed using A-scan ultrasonography (OTI-Scan 1000, Ophthalmic Technologies Inc., Downsview, Ontario, Canada). This ultrasonography system used the acoustic velocities for humans eyes to compute the separations between acoustic interfaces (cornea and lens: 1641 m/s, aqueous and vitreous: 1532 m/s) (Byrne & Green, 2002). Ten independent measurements were made along the normal to the corneal apex using a 13 MHz transducer, and the readings were averaged.

Sub-foveal choroidal thickness in the DL-controls and DL-FD monkeys was measured using a spectral-domain, optical coherence tomography system (SD-OCT; Spectralis, Heidelberg, Germany) following the methodology described by Hung *et al.* (Hung *et al.*, 2018). In brief, images acquired using the OCT "Enhanced-Depth Imaging" mode were manually segmented using a customized Matlab program (2019a, MathWorks, Natick, MA, USA). Choroidal thickness, defined as the distance between Bruch's membrane and the outer choroidal border, was measured perpendicular to Bruch's membrane. The average choroidal thickness of a 300-micron region (adjusted for retinal magnification) (Patel *et al.*, 2017) centered at the deepest point of the macula depression (the foveola) was compared interocularly over the course of the experiment. The thickness measures were highly repeatable. The mean (±SD) absolute thickness differences obtained from repeated OCT scans obtained at the same

measurement session was 5.07 ± 3.86 μm, about 3% of the mean choroidal thickness in dim-light monkeys.

It has been found that visual manipulations commonly used to induce experimental ametropias consistently and predictably produced choroidal thickness changes. Specifically, in chicks, dramatic choroidal thickening or thinning can occur in response to myopic and hyperopic defocus, respectively, to reduce the presenting optical error (Wallman *et al.*, 1995; Wildsoet & Wallman, 1995). In primates, defocus-induced choroidal thickness changes are qualitatively similar to those observed in chicks, but are much smaller in magnitude and thus have little direct effect on the eye's refractive state (Hung *et al.*, 2000; Troilo *et al.*, 2000a). In this respect, choroidal thickness changes could reflect retinal processing of defocus signals. It is useful particularly for the observations of recovery from FDM under dim light, in which the visual signals that drive the recovery (Qiao-Grider *et al.*, 2004; Schaeffel & Howland, 1991; Wildsoet & Schmid, 2000) might not be sufficiently strong to alter refractive development (Gottlieb *et al.*, IOVS, 1991; 32: ARVO abstract; She *et al.*, 2020).

All rearing and measurement procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the University of Houston's Institutional Animal Care and Use Committee.

3.2.5 Statistical methods

Data were analyzed cross-sectionally and longitudinally. Cross-sectional analyses were performed for data obtained at the onset of the dim-light-diffuser period, the onset of the dim-light-recovery period (i.e., the end of dim-light-diffuser period), and the end of dim-light-recovery period. Paired and student's t-tests were employed for the between-eye and between-group analyses of refractive errors and ocular parameters, respectively.

Mixed-effect models were used to compare the longitudinal refractive and vitreous chamber development between DL-FD and NL-FD groups (Sophia Rabe-Hesketh & Skrondal, 2012). Because the age-related changes of most ocular parameters are typically curvilinear, the models were constructed as a 2nd order polynomial function of age in a forward selection manner. The statistics for the quadratic effect coefficients were reported only when the effects were statistically significant.

Finally, Pearson correlation and linear regression were used to characterize the relationship between refractive error and ocular parameters. Specifically, we were interested in whether the refractive errors were related to vitreous chamber elongation, the biometrical determinant of most visually induced ametropias in rhesus monkeys. In this respect, the vitreous chamber to corneal radius ratio (VC/CR ratio) was employed for the correlational and regression analyses. In comparison to using absolute vitreous chamber depth, this metric reduces the noise in correlational and regression analyses because it accounts for between-animal differences in the contribution of the cornea to refractive development (She *et al.*, 2020).

All statistical procedures were performed using STATA (MP 14; StataCorp, College Station, TX, USA) at a significance level of 0.05.

3.3 **Results**

3.3.1 Refractive developments in the dim-light-diffuser period

The baseline refractive errors and ocular parameters for the two diffuser groups are compared in Table 3-1. At the onset of the experiment, the DL-FD monkeys were slightly older (2 days) than the NL-FD monkeys ($p = 0.02$). Despite this difference, the refractive errors and ocular parameters of the DL-FD monkeys were similar to those in NL-FD monkeys. The refractive

errors in the two eyes of the DL-FD monkeys were also similar; however, the treated eyes of the NL-FD monkeys were slightly less hyperopic than their control eyes ($t(15) = -2.55$, $p = 0.02$). Because no significant between-eye difference in vitreous chamber depth was observed (Table 3-1), we speculate that this between-eye refractive error difference might reflect measurement variabilities. Finally, the absolute magnitude of the interocular differences in refractive error was similar between the two groups. Considering the degree of anisometropia that can be induced by diffusers, these baseline interocular differences were negligible.

After the onset of the dim-light-diffuser period, most of the DL-FD subjects showed obvious alterations in the course of refractive development that reflected the monocular nature of the diffuser treatment (Figure 3-2, open/filled red symbols). The exceptions were monkeys 672 and 671 (Figure 3-2A and B), both of which remained isometropic despite experiencing monocular form deprivation. Specifically, monkey 672 (Figure 3-2A) showed a slight increase in hyperopia in the two eyes over time, whereas monkey 671 exhibited moderate reductions in hyperopia that were within the range seen in normally emmetropizing monkeys (Figure 3-2B). The interocular differences in refractive error remained small and stable for these two monkeys throughout the dim-light-diffuser period. For the remaining subjects, large reductions in hyperopias took place in their form-deprived eyes (Figure 3-2C – G). For monkey 674, the early myopic refractive shift appeared to stabilize at approximately 90 days of age (Figure 3-2C), but for monkeys 670, 673, 675, and 676 (Figure 3-2D – G), the myopic alterations continued and eventually resulted in substantial absolute myopias.

With the exception of monkey 671 (Figure 3-2B), the treatment regimen also altered the normal refractive development that was anticipated in the fellow control eyes of the DL-FD monkeys. Instead of exhibiting age-related reductions of hyperopia, these eyes either roughly maintained the same amount of hyperopia (monkeys 672, 674 and 670, Figure 3-2A, C, and D)

or showed greater reductions in hyperopia than what was typically associated with normal emmetropization, and later became more myopic than normal-control monkeys (monkeys 673, 675 and 676, Figure 3-2E, F and G). Note that the relative myopic alterations in the fellow eyes were also very different from our previous observations, in which monkeys reared with unrestricted vision under dim light developed age-related increases, rather than decreases, in hyperopia (She *et al.*, 2020).

Dim light appeared to affect the time course for refractive development in the control eyes, but not the diffuser-treated eyes. Figure 3-3 illustrates the average changes (±SD) in refractive error for the DL-FD and NL-FD monkeys during the diffuser-rearing period. The treated eyes of both diffuser groups showed greater reductions in hyperopia than their respective control eyes and the eyes of normal-control monkeys; however, no significant differences were observed between the treated eyes of the two diffuser groups (Figure 3-3A and B), indicating that dim-light rearing did not affect the development of FDM. As for the control eyes, the time course for the age-related reductions in hyperopia in the DL-FD monkeys appeared to be initially slower than that of the NL-FD monkeys, but later accelerated towards the end of the dim-light-diffuser period ($z = -3.73$, $p < 0.01$ for the quadratic effect coefficients). In addition, the standard deviations of the changes in control-eye refractive error were greater in the DL-FD monkeys than in NL-FD monkeys (Figure 3-3A and B), a reflection of the individual variability of their refractive development. Despite the variability in the later stages of diffuser rearing, the average fellow-eye refractive changes in the DL-FD monkeys were within the 95% confidence limits for the normal control monkeys (Figure 3-3B).

Dim-light rearing did not appear to affect the magnitude of the refractive changes induced by form deprivation. Figure 3-4. shows the refractive errors obtained at the end of the diffuser-rearing period for the treated (filled symbols) and control eyes (open symbols) of individual

DL-FD and NL-FD monkeys. At the end of the diffuser-rearing period, NL-FD monkeys exhibited various degrees of myopia in their treated eyes in comparison to the NL-controls (NL-FD vs. NL-controls: -1.30 ± 4.28D vs. + 2.34 ± 1.07D, t (55) = -5.1, $p < 0.001$), whereas their control eyes were relatively more hyperopic (+ 3.18 ± 1.68D vs. +2.34 ± 1.06D, t (55) = 2.24, $p = 0.03$). Except for the control eye of one DL-FD monkey that fell below the 95% confidence limits for normal-control monkeys, the ranges of treated- and control-eye refractive errors in the DL-FD and NL-FD monkeys were very similar. There were no significant differences between the DL-FD and NL-FD monkeys in the mean refractive errors for either the treated or control eyes. In addition, the degree of myopic anisometropias in the NL-FD and DL-FD groups was statistically the same (mean ± SD of IOD, DL- vs. NL-FD: -3.88 ± 3.26 D vs. -4.48 ± 3.73 D). On the other hand, due to the excessive reductions in hyperopia in the fellow eyes of DL-FD monkeys 673, 675, and 676, the control eyes of the DL-FD monkeys appeared less hyperopic than those of the DL-control monkeys, although the difference was not statistically significant (+3.70 ± 1.36D vs. +2.01 ± 2.78D) (She *et al.*, 2020).

3.3.2 Ocular parameters in the dim-light-diffuser period

At the end of diffuser-rearing period, the corneal powers, anterior chamber depths, and lens thicknesses were similar between DL-FD and NL-FD monkeys (Table 3-1) and were consistently within the ±2 SD range of the normal-control monkeys (Figure 3-5A – C). In addition, there were no significant between-eye differences in these ocular components.

In agreement with the refractive outcomes noted above, the course of treated-eye vitreous chamber depth development was similar in the DL-FD and NL-FD monkeys ($z = 1.45$, $p = 0.15$), indicating that dim-light rearing did not affect the ocular axial elongation that associates with FDM. At the end of the dim-light-diffuser period, vitreous chamber depths of the DL-FD monkeys were greater in their form-deprived eyes than in their control eyes (t (6) = 3.22, p =

0.02), but there were no significant differences between the DL-FD and NL-FD monkeys for either the treated or control eyes (Figure 3-5D). As illustrated in Figure 3-6, at the end of the dim-light-diffuser period, the refractive errors of the DL-FD monkeys were inversely correlated with their vitreous chamber to corneal radius ratios (VC/CR ratio; $r = -0.97$, $p < 0.01$), suggesting that vitreous chamber depth remained the primary determinant of the observed refractive errors after accounting, at least in part, for the possible influence of individual differences in corneal power.

3.3.3 Sub-foveal choroidal thicknesses in relation to the interocular differences in refractive errors

At the onset of the experiment, the choroidal thicknesses in the treated (average ± SEM thickness: 107.80 ± 6.77 μm) and control eyes of the DL-FD monkeys (108.38 ± 6.88 μm) were similar. During the dim-light-diffuser period, the fellow control eyes of all of the DL-FD monkeys exhibited age-associated increases in choroidal thickness. Specifically, sub-foveal choroidal thickness in the control eyes increased in a roughly linear manner after the onset of the dim-light-diffuser period (0.32 micron/day, $z = 2.49$, $p = 0.01$) and these longitudinal changes did not differ significantly from those observed in the DL-control monkeys. At the end of the dim-light-diffuser period, the average (±SEM) choroidal thickness in the fellow control eyes of the DL-FD monkeys was similar to the binocularly averaged choroidal thickness observed in the age-matched, DL-control monkeys (DL-FD control eyes vs. DL-controls: 150.76 ± 13.21 μm vs. 167.11 ± 6.24 μm) (She *et al.*, 2020) and were not significantly different from the average values in the two eyes of the NL-controls (147.18 ± 6.44 μm).

The interocular differences in refractive error observed in the DL-FD monkeys were associated with interocular differences in choroidal thickness (Figure 3-7.). For the two DL-FD monkeys that failed to develop myopic anisometropia during the diffuser-rearing period,

choroidal thicknesses were either similar in both eyes or slightly thicker in the deprived eyes (Figure 3-7.A and B). However, in each of the 5 monkeys that developed obvious myopic anisometropias, the treated-eye choroids were consistently thinner than the choroids in the fellow control eyes, at least during the early stages of diffuser wear (i.e., about the first 30 days of diffuser wear). These initial responses were qualitatively similar to those observed in deprived eyes of chicks, tree shrews, macaque monkeys, and marmosets that were reared under typical laboratory lighting (Troilo, Nickla, and Wildsoet, 2000a; Siegwart Jr. and Norton, 1998; Wallman *et al.*, 1995; Hung, Wallman, and Smith III, 2000). Subsequently, the relative choroidal thinning in the deprived eyes of the DL-FD monkeys quickly slowed; as the diffuser treatment continued, the interocular differences in choroidal thickness were then either roughly maintained (e.g., Figure 3-7.C, D, E) or decreased (Figure 3-7.F and G). As a consequence, the average (±SEM) choroidal thickness in the treated and control eyes of the DL-FD monkeys were not significantly different at the end of dim-light-diffuser period (diffuser-treated eyes vs. control eyes: 149.53 ± 48.28 μm vs. 150.76 ± 34.96 μm). The temporal pattern of the relative choroidal thickness changes in the DL-FD monkeys suggested that the relative choroidal thinning in the treated eyes was associated with the ocular responses that eventually led to FDM.

3.3.4 Changes in refraction during the recovery period and their relationship with choroidal thickness

Several observations during the recovery period indicated that the dim-light regimen interfered with the normal regulation of the eye's refractive state. First, the two subjects that did not develop FDM continued to show atypical refractive development after the removal of diffusers. Specifically, monkey 672 (Figure 3-2A) remained isometropic, but became progressively more hyperopic in both eyes, i.e. both eyes failed to compensate for the increasing amounts of

hyperopic defocus. For monkey 671 (Figure 3-2B), the treated eye maintained the low degree of hyperopia that was observed at the end of the diffuser treatment period, but its control eye showed progressive myopic changes that resulted in an obvious hyperopic anisometropia, suggesting that the control eye was not responding appropriately to myopic defocus. Most obviously, for the DL-FD subjects that developed FDM, the anticipated recovery from myopic anisometropias was not consistent. Although the myopic shifts in the treated eyes of monkeys 673, 675, and 676 (Figure 3-2E – G) stopped after the removal of diffusers, these eyes did not show the rapid hyperopic shift that was observed in monkeys 674 and 670 (Figure 3-2C and D), thus the degree of their myopic anisometropias did not decrease over the recovery period.

In contrast to the DL-FD monkeys, NL-FD monkeys consistently recovered from FDM when unrestricted vision was restored. Figure 3-8 compares the recoveries from FDM under normal and dim ambient lightings. In panel A, the recovery data from every NL-FD monkey that had at least 1.5 D of myopic anisometropia at the end of diffuser rearing (filled symbols) are plotted as a function of age. In addition, recovery data from all monkeys previously reared under "normal light" with weaker diffusers (Smith III & Hung, 2000) or with LP diffusers combined with daily brief periods of unrestricted vision (Smith III *et al.*, 2002) and that also exhibited at least 1.5 D of myopic anisometropia are also included (thin solid lines). These data show that the time course of recovery varies with the initial degree of FDM, but given sufficient time even animals with large myopic anisometropias exhibit substantial degrees of recovery (Qiao-Grider *et al.*, 2004; Smith III *et al.*, 2017). Specifically, as illustrated in panel C, which shows the initial degree of myopic anisometropia and that was observed after 156 ± 29 days of recovery for individual animals, all but 1 of the 23 diffuser-reared subjects that developed FDM (a NL-FD subject; yellow symbol in panels A and C) showed systematic reductions in myopic anisometropia following the onset of unrestricted vision. However, for the DL-FD monkeys, only two animals showed similar recovery patterns at rates that were comparable to those of

the NL-FD monkeys (Figure 3-8B, red symbols); the other three NL-FD monkeys failed to show any systematic reductions in the degree of myopic anisometropia (Figure 3-8B, blue symbols), even though the degrees of their myopic anisometropias at the start of the recovery period were similar to those of the two DL-FD subjects that recovered and were within the range of anisometropias where recovery consistently occurred under normal lighting levels (Figure 3-8C). A Fisher's exact test found that dim-light-reared monkeys appeared to be less likely to exhibit a similar recovery pattern (two-sided Fisher's exact $p = 0.08$). The average changes in myopic anisometropias in the DL-FD group were smaller than those in the NL-FD group ($+0.44 \pm 2.99$D vs. $+2.98 \pm 1.53$D, $p = 0.046$), despite the fact that the average length of recovery period was longer in the DL-FD group (181 ± 10 vs. 156 ± 29 days).

The recovery from monocularly induced FDM in monkeys is normally correlated with interocular differences in vitreous chamber elongation rate (Smith III & Hung, 2000; Troilo & Nickla, 2005). As illustrated in the left column of plots in Figure 3-9, in which the developmental courses for the IODs in refractive error and vitreous chamber depth (treated eye – fellow control eye values) are plotted for individual DL-FD monkeys, the refractive changes that took place after removing the diffusers were associated with alterations in vitreous chamber elongation rate, and the resulting refractive outcomes were axial in nature. For example, the DL-FD monkey that remained isometropic (672, Figure 3-9A) showed no systematic changes in the relative balance of the vitreous chamber depths in its two eyes. Monkey 671 (Figure 3-9B), which developed a hyperopic anisometropia during the recovery period, concurrently exhibited a relatively shallower vitreous chamber in its treated eye (i.e., negative IODs). The reductions in the diffuser-induced axial myopic anisometropias in monkeys 674 and 670 were accompanied by synchronized reductions in the interocular differences in vitreous chamber depth (Figure 3-9C and D). In contrast, the DL-FD monkeys that did not show systematic signs of recovery (monkeys 673, 675, and 676; Figure 3-9E, F

and G) also failed to show obvious changes or reductions in the interocular differences in vitreous chamber depth.

The right column of Figure 3-9 illustrates the changes in choroidal thickness during the recovery period for the treated and control eyes of DL-FD monkeys. For the monkeys that did not develop FDM (monkeys 672 and 671), the choroidal thickness changes were roughly symmetrical in their two eyes (Figure 3-9H and I). For monkeys that developed FDM, relative choroidal thickening in the treated eye, which was expected in response to myopic defocus, occurred exclusively in the two animals that showed obvious signs of recovery. Specifically, the choroids in the treated eyes of monkeys 674 and 670 thickened (~20 μm) rapidly after the restoration of unrestricted vision, whereas the choroids of their control eyes thinned (Figure 3-9J and K). These rapid initial relative changes in response to the onset of unrestricted vision, which agree well with the existing knowledge that myopic defocus causes an increase in choroidal thickness (Hung *et al.*, 2000; Troilo *et al.*, 2000; Wallman *et al.*, 1995; Wildsoet & Wallman, 1995), were not observed in monkeys that did not show signs of recovery (673, 675, and 676, Figure 3-9L, M and N).

3.4 **Discussion**

Our results showed that dim-light rearing increased the variability of control eye refractive development, but it did not change refractive development in the form-deprived eyes, thus had no significant effect on the degree of FDM and the underlying axial elongation. However, dim-light rearing interfered with the anticipated recovery from FDM and promoted the development of abnormal refractive errors during the recovery period in some fellow control eyes. The failure to recover from FDM was accompanied by the absence of defocus-related choroidal

thickening in the treated eyes, suggesting that the response to existing optical defocus signals might be impaired under dim light.

3.4.1 Comparison to previous studies

In monocularly form-deprived chicks, reduced ambient lighting levels similar to those employed in this study did not alter the course of FDM in the treated eyes or emmetropization in the fellow control eyes (Ashby *et al.*, 2009). In the present study, dim-light rearing also did not prevent nor exacerbate the development of FDM in the treated eyes of DL-FD monkeys, despite the fact that the length of our daily exposure and the total length of our treatment regimen were both much longer than those in the Ashby *et al.* investigation. Together, these findings indicate that, in the absence of meaningful visual feedback, reduced ambient lighting does not enhance deprivation-induced myopia and thus does not appear to be myopiagenic, per se. Moreover, these results suggest that, in order for ambient lighting levels to influence the phenomenon of FDM, lighting levels must be above our dim lighting levels and above typical indoor lighting levels (Ashby *et al.*, 2009; Chen *et al.*, 2017; Karouta & Ashby, 2015; Siegwart Jr. *et al.*, 2012; Smith III *et al.*, 2012).

On the other hand, dim-light rearing altered the course of refractive development and increased the refractive variability in the fellow control eyes of our DL-FD monkeys. In particular, the fellow-eye myopic changes observed in three DL-FD monkeys that developed myopic anisometropia were very different not only from the relative hyperopic changes found in DL-control monkeys (She *et al.*, 2020), but also from those observed in the fellow eyes of the NL-FD monkeys (Hung, Arumugam, She, *et al.*, 2018; Smith III & Hung, 2000). It is possible that these relative myopic changes came about because dim light influenced the impact of potential interocular factors associated with monocular form deprivation, which have been reported in both chickens and monkeys reared under more typical ambient lighting levels

(Bradley *et al.*, 1999; Raviola & Wiesel, 1985; Schmid & Wildsoet, 1996; Smith *et al.*, 1987; Smith III & Hung, 2000; Wildsoet & Wallman, 1995). Considering that none of our DL-control animals developed relative myopias, the myopic tendencies observed in the control eyes suggested that low intensity ambient lighting could be myopiagenic under certain conditions. For the DL-FD monkeys in particular, low ambient lighting was myopiagenic for the control eyes when the treated eye developed vision-induced myopia. Similar interocular effects were not observed in monocularly form-deprived chicks reared in dim ambient lighting, possibly because the length of the observation period was too short (Ashby *et al.*, 2009).

The nature of the observed refractive errors is an important consideration for the discussion of mechanisms related to the refractive effects of ambient lighting levels. Although form-deprivation in typical laboratory lighting (Gottlieb, Fugate-Wentzek, & Wallman, 1987. Also see: Hayes *et al.*, 1986) and in dim lighting (Cohen *et al.*, 2011, 2012) has been reported to induce corneal curvature changes in chickens, neither Ashby *et al.*'s (Ashby *et al.*, 2009) study of FDM in chickens (again possibly due to the short treatment duration) nor the present study observed systematic alterations in corneal powers. Specifically, in the present study, several observations (such as changes in ocular biometric parameters, correlation between refraction and the VC/CR ratio, and the relative choroidal thinning in the treated eyes) indicate that the FDM observed in the dim-light-reared monkeys maintained its widely conserved axial nature (chicks: Gottlieb, Joshi, and Nickla, 1990; Hayes *et al.*, 1986; Troilo *et al.*, 1995; Wallman and Adams, 1987; Wallman, Turkel, and Trachtman, 1978; Mouse: Schaeffel *et al.*, 2004; tree shrews: Norton and Rada, 1995; guinea pigs: Howlett & McFadden, 2006; non-human primates: Smith III *et al.*, 2000; Troilo and Nickla, 2005) and was qualitatively similar to that observed under both typical indoor lighting levels (Smith III & Hung, 2000) and elevated ambient lighting levels (Smith III *et al.*, 2012).

3.4.2 Possible explanations for the absence of effects on FDM

Light-intensity dependency of refractive error has been observed in chicks reared with form-deprivation (Karouta & Ashby, 2015) and unrestricted vision (Cohen *et al.*, 2011, 2012), suggesting that light levels could be quantitatively translated into biological signals that affect eye growth. In this regard, retinal dopamine has been suggested to be a candidate molecule for mediating light intensity effects, although the exact mechanism is not well understood (Feldkaemper & Schaeffel, 2013; Norton & Siegwart Jr., 2013; Zhou *et al.*, 2017). Notably, Norton and Siegwart suggested that lighting levels might act as a continuous factor of influence for refractive error through light-induced changes in retinal dopamine levels (Norton & Siegwart, 2013). In comparison to the phenomena of lens compensation and possibly emmetropization, this hypothesis appears more reasonable for FDM, not only because the form-deprived eyes are unable to use the presenting refractive errors to control refractive development and ocular growth (T. W. Park *et al.*, 2003), but also because retinal dopamine levels have been found to be inversely associated with the degree of myopic changes and axial elongation (Stone *et al.*, 1989).

Although the speculation noted above predicts more FDM as dim light potentially reduces retinal dopamine levels, the previous investigation in chicks (Ashby *et al.*, 2009) and our observations in infant monkeys both indicated otherwise. In addition, although Cohen *et al.*, showed that long-term chronic exposure to dim light caused reduced retinal dopamine levels (and more myopia) in emmetropizing chicks (Cohen *et al.*, 2012), there is no evidence that dim light could further reduce the retinal dopamine levels in form-deprived eyes. On the country, the adaptive (Dubocovich *et al.*, 1985; Porceddu *et al.*, 1987) and multifactorial retinal dopaminergic system (Brainard & Morgan, 1987; Cohen, Iuvone, & Neff, 1981; Hadjiconstantinou, Cohen, & Neff, 1983; Iuvone *et al.*, 1978; Iuvone & Rauch, 1983;

Marshburn & Iuvone, 1981; Proll, Kamp, & Morgan, 1982; Stone *et al.*, 1989) might be able to compensate for the decreases in dopamine production induced by the relative drop in absolute ambient illuminance (~50 lux vs. ~500 lux), at least for eyes that are permitted unrestricted vision. In this regard, Landis *et al.* have shown that, whereas the level of 3,4-dihydroxyphenylacetic acid (DOPAC) in the retina showed light-intensity dependency, rearing with chronic exposures to reduced ambient lighting (0.005 lux and 50 lux) did not have significant effects on retinal dopamine levels in mice in comparison to higher ambient lighting (Landis *et al.*, 2019). Based on the available evidences, the refractive effect of reduced ambient lighting levels does not appear to be mediated by quantitative changes in retinal dopamine levels.

It is also possible that dim light did not have a significant refractive effect because the ocular elongation responses in the treated eyes of the DL-FD monkeys were saturated. This rate limitation might arise, for example, from the active biochemical changes that take place in the sclera (scleral remodeling) (Gentle *et al.*, 2003; McBrien *et al.*, 1991, 2001) and/or from the resulting changes in scleral biomechanical properties (Phillips *et al.*, 2000; Siegwart Jr. & Norton, 1999). In addition, rate limitations could also occur because the visual trigger for FDM had reached the maximum myopiagenic strength when the deprivation paradigm was employed under either reduced or normal lighting levels. The myopiagenic visual trigger associated with form deprivation has been implied to be the degradation in image contrast (Bartmann & Schaeffel, 1994), of which the perception might be worse under dim light. Although some (Bartmann & Schaeffel, 1994; Smith III & Hung, 2000), but not all (Tran *et al.*, 2008), studies suggested that FDM is a graded phenomenon, a limited operating range for such a "dose-response" relationship appears to exist. For example, Tran *et al.* showed that a quantitatively similar amount of FDM and axial elongation could be induced by diffusers with different nominal strengths (Tran *et al.*, 2008). This possible operating range limitation suggests that

dim light might not be able to exacerbate FDM by increasing the myopiagenic strength of certain form-deprivation paradigms. However, it also suggests that the potential myopiagenic effects associated with low lighting levels could become obvious if weaker diffusers are employed. In this respect, our findings cannot exclude the possibility that dim light is a risk factor of myopiagenesis.

Regardless of the underlying events, the results from both chickens and monkeys consistently suggest that ambient lighting levels only affect the course of FDM at lighting levels above those that associate with typical indoor environments.

3.4.3 Implications of observations made during the recovery period

As observed in other species (Howlett & McFadden, 2006; Siegwart & Norton, 1998; Wallman & Adams, 1987), rhesus monkeys reared under typical laboratory lighting consistently recovered from FDM (Qiao-Grider *et al.*, 2004). On the contrary, only two of the DL-FD subjects that developed FDM exhibited signs of recovery, despite the fact that the progressive myopic changes in these subjects quickly stopped after the diffusers were removed. Considering that the FDMs in the DL-FD monkeys were primarily attributed to diffuser wear rather than dim-light rearing, the cessation of myopia progression after the onset of recovery period was probably not surprising. However, it is less clear whether the cessation of myopia progression was a result of diffuser withdrawal or was related to the presence of myopic defocus (Wildsoet & Schmid, 2000).

In this regard, our choroidal thickness measurement suggested that the absence of obvious signs of recovery might be associated with a failure to detect and/or respond to the presence of the myopic errors. At typical laboratory lighting levels, increases in choroidal thickness have been observed in chicks (Wallman *et al.*, 1995), guinea pigs (Howlett & McFadden, 2006), tree

shrews (Siegwart Jr. & Norton, 1998) and non-human primates (Hung *et al.*, 2000; Troilo *et al.*, 2000a) recovering from induced myopic ametropias. In the current study, we found that relative increases in choroidal thickness were observed only in the treated eyes of monkeys that showed signs of recovery, whereas monkeys that did not recover showed qualitatively different choroidal thickness change patterns. Because the recovery from FDM under typical ambient lighting has been shown to be driven by the myopic defocus in the treated eyes (Schaeffel & Howland, 1991; Wildsoet & Schmid, 2000), the absence of appropriate choroidal thickness alterations suggests that the treated eyes of these DL-FD monkeys might have failed to detect and/or process the myopic defocus.

The manner in which dim light interfered with the recovery from FDM and the abnormal refractive errors observed in some animals during the recovery period were somewhat analogous to the failure of emmetropization observed in DL-control monkeys (She *et al.*, 2020). In both cases, the probability that alterations in ocular growth would compensate for an existing refractive-error signal was decreased. Although we had previously speculated that some DL-control monkeys were unable to emmetropize because the hyperopic errors were not sufficiently strong to trigger and/or maintain emmetropization (She *et al.*, 2020), the absence of consistent recovery responses in monkeys that experienced large and sustained degrees of FDM suggests that the functional state of the emmetropization process was impaired. In support of this view, the two DL-FD monkeys that did not initially develop FDM also exhibited abnormal refractive development after the removal of the diffuser lenses, suggesting that the emmetropization process did not respond to the presenting defocus signals. Together, these observations indicated that low ambient lighting levels could be a potential risk factor for refractive anomalies in primates (She *et al.*, 2020).

3.4.4 Conclusions and implications

The observations that dim-light rearing did not affect the time course or degree of FDM in rhesus monkeys and chicks suggests that low intensity ambient lighting, by itself, is not a strong environmental enhancer of visually induced myopia. In view of our previous observations in DL-control monkeys (She *et al.*, 2020), the inability of dim light to alter the unregulated, "intrinsic" ocular growth rate that underlies FDM suggests that dim light primarily affects the mechanisms that operate to eliminate defocus errors. The reduced probability to recover from FDM and the absence of relative choroidal thickening that normally is produced by myopic defocus provide support for this speculation. Due to our small sample size and the variabilities in the final degree of FDM and its recovery, further studies on the compensating responses to imposed defocus are required to confirm the association between dim light and possible defects in the mechanisms that process defocus signals.

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Tables and Figures

Table 3-1. Refractive error and ocular parameters for the FD monkeys

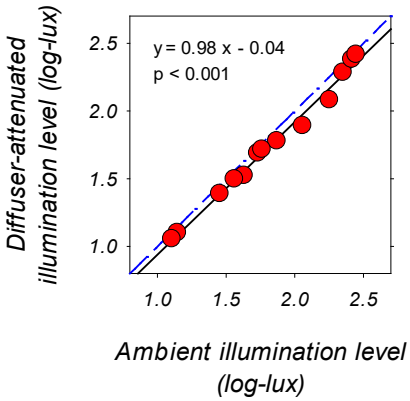
	Baseline				End of the dim-light-diffuser period			
	DL-FD (25 ± 3 days)[1]		NL-FD (23 ± 2 days)		DL-FD (154 ± 7 days)		NL-FD (149 ± 23 days)	
	Treated eye	Control eye	Treated eye	Control eye	Treated eye	Control eye	Treated eye	Control eye
Refractive error (mean ± SD. D)	3.73 ± 1.25	3.78 ± 1.23	4.13 ± 1.29 *	4.29 ± 1.28	-1.87 ± 4.81*	+2.01 ± 2.78	-1.30 ± 4.28 *	+3.18 ± 1.68
Corneal power (mean ± SD, D)	62.16 ± 0.97	61.93 ± 0.98	61.14 ± 1.34	60.98 ± 1.22	55.73 ± 0.72	56.05 ± 0.78	55.98 ± 1.52	55.45 ± 1.57
Anterior chamber depth (mean ± SD, mm)	2.38 ± 0.15	2.4 ± 0.15	2.66 ± 0.16	2.61 ± 0.28	2.92 ± 0.1	2.99 ± 0.11	3.12 ± 0.24	3.14 ± 0.24
Lens Thickness (mean ± SD, mm)	3.72 ± 0.08	3.72 ± 0.06	3.60 ± 0.15	3.62 ± 0.2	3.72 ± 0.13	3.67 ± 0.14	3.57 ± 0.15	3.59 ± 0.14
Vitreous chamber depth (mean ± SD, mm)	8.44 ± 0.29	8.42 ± 0.3	8.62 ± 0.27	8.65 ± 0.26	10.76 ± 0.75 *	9.93 ± 0.5	10.60 ± 0.92 *	9.89 ± 0.58

[1]. Significant, but negligible, between-group difference in starting age
* Significant interocular difference.

Refractive error and ocular parameters at the onset and end of the dim-light-diffuser period. Asterisks denote significant interocular differences.

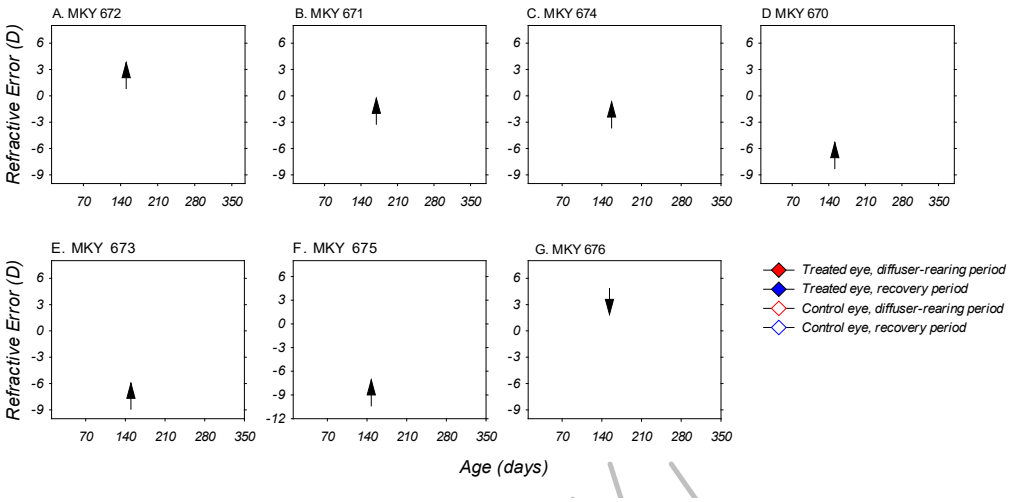
There were no significant between-group differences in refractive error or any of the ocular parameters at either time point.

Figure 3-1. Light attenuation by the diffusers



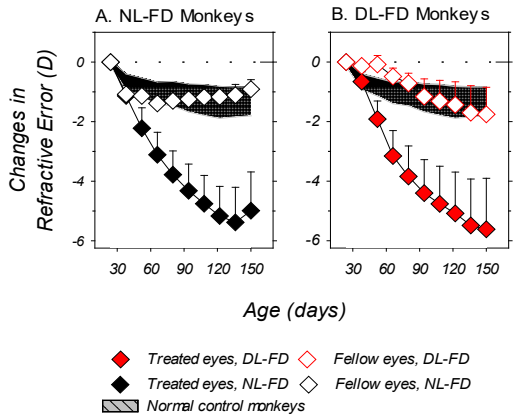
Diffuser-attenuated illumination levels plotted as a function of the corresponding ambient illumination levels on common logarithmic scales. The ambient lighting levels were within the dim-to-normal range employed in the present study. The blue dashed line represents zero diffuser-induced light attenuation, whereas the black regression line represents the linear relationship between diffuser-attenuated and unfiltered ambient illumination levels. To measure the diffuser-attenuated illumination levels, a piece of diffuser foil was attached to a zero-power carrier lens, of which the peripheral area was taped to block stray lights. The center of the diffuser lens was then placed perpendicular to the measurement axis of a spectrophotometer at a 14 mm distance (CL-500A, Konica Minolta Sensing Americas, Inc. NJ, USA). For the measurement of corresponding ambient illumination levels, another taped carrier lens with a clear center of equal size was used in place of the diffuser-attached lens.

Figure 3-2. Refractive developments for individual DL-FD monkeys



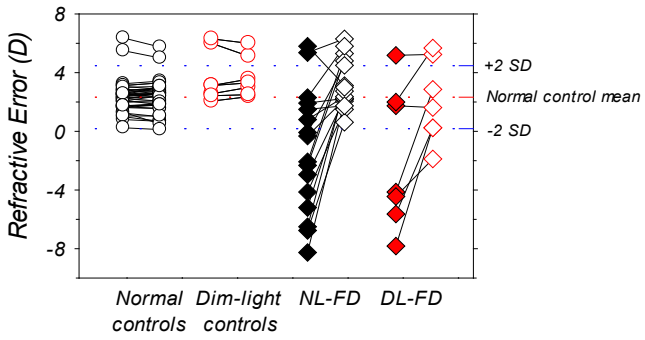
Spherical-equivalent, spectacle-plane refractive corrections plotted as a function of age for individual DL-FD subjects. The diffuser-treatment and recovery periods (see section 3.3.4) are highlighted in red and blue, respectively. Black arrows: the last day of the dim-light-diffuser period and onset of the recovery period; filled symbols: treated eye; open symbols control eyes; thin grey lines: the refractive errors for the right eyes of 41 age-matched, normal-light-reared control monkeys.

Figure 3-3. Mean refractive changes



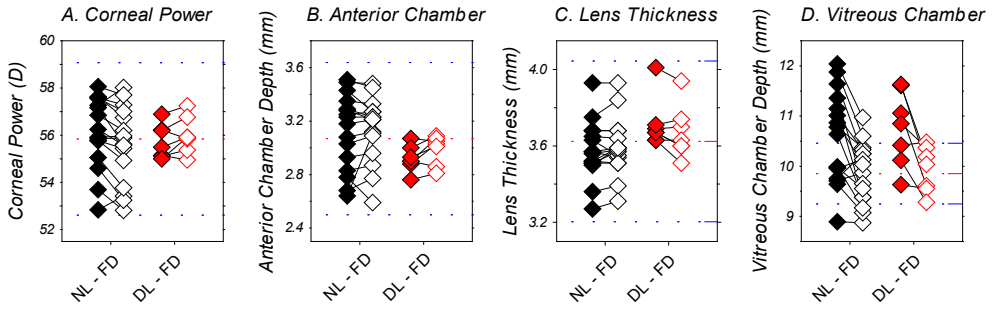
Mean (+SD) refractive-error changes relative to baseline plotted as a function of age. The filled and open symbols represent the treated and control eyes of the NL-FD (Panel A) and DL-FD (Panel B) monkeys, respectively. The shaded areas represent the 95% confidence range for the changes in refractive error in the right eyes of the age-matched, normal-control monkeys.

Figure 3-4. Refractive error at the end of the dim-light-diffuser period



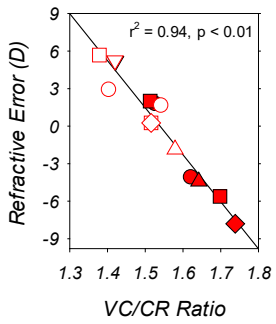
Spherical-equivalent, spectacle-plane refractive corrections for individual animals at the end of the dim-light-diffuser period. The filled and open diamonds represent the treated and control eyes of the DL-FD and NL-FD monkeys, respectively. The circle symbols represent the two eyes of the NL-control (open circles) and DL-control monkeys (open red circles), respectively. The red and blue dashed lines represent the mean and ±2 SD from the mean of the normal-control monkeys.

Figure 3-5. Ocular parameters at the end of the dim-light-diffuser period



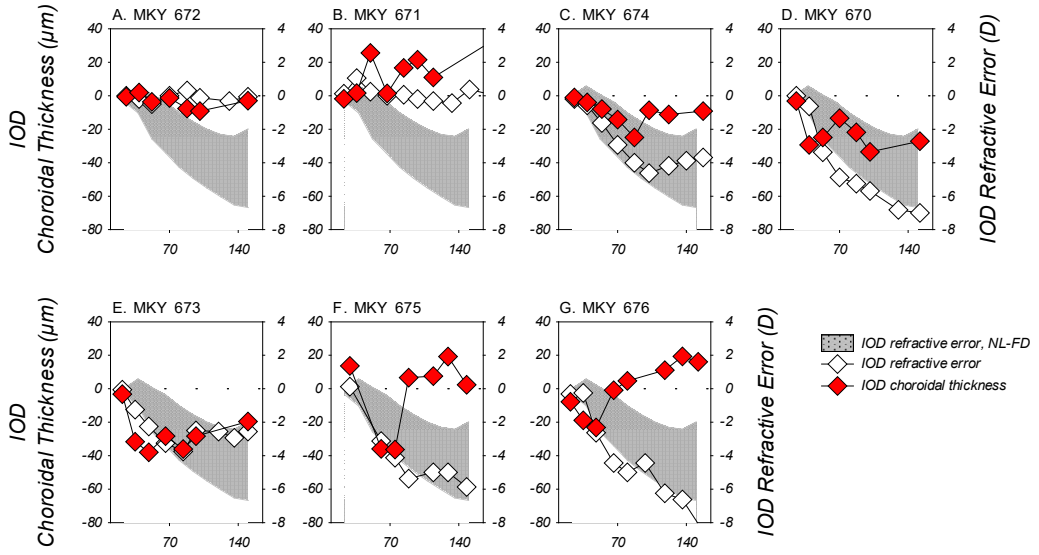
Ocular parameters measured at the end of the diffuser-rearing periods for individual form-deprived monkeys. The filled and open symbols represent the treated and control eyes of the DL-FD (red) and NL-FD monkeys (black), respectively. The red and blue dashed lines represent the mean and ±2 SD from the mean for each respective ocular parameter for the normal-control monkeys.

Figure 3-6. Correlation between VC/CR ratio and refractive error



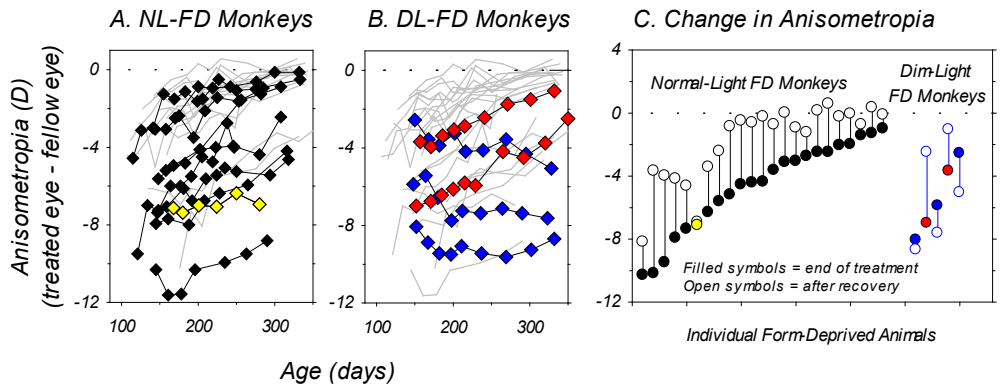
Spherical-equivalent, spectacle-plane refractive corrections plotted as a function of the vitreous chamber to corneal radius ratio (VC/CR ratio) for individual DL-FD monkeys obtained at the end of the dim-light-diffuser period. Filled and open symbols represent the treated and control eyes, respectively. Vitreous chamber depth and the radius of corneal curvature for the calculation of VC/CR ratio were specified in millimeters.

Figure 3-7. interocular differences in refractive error and choroidal thickness



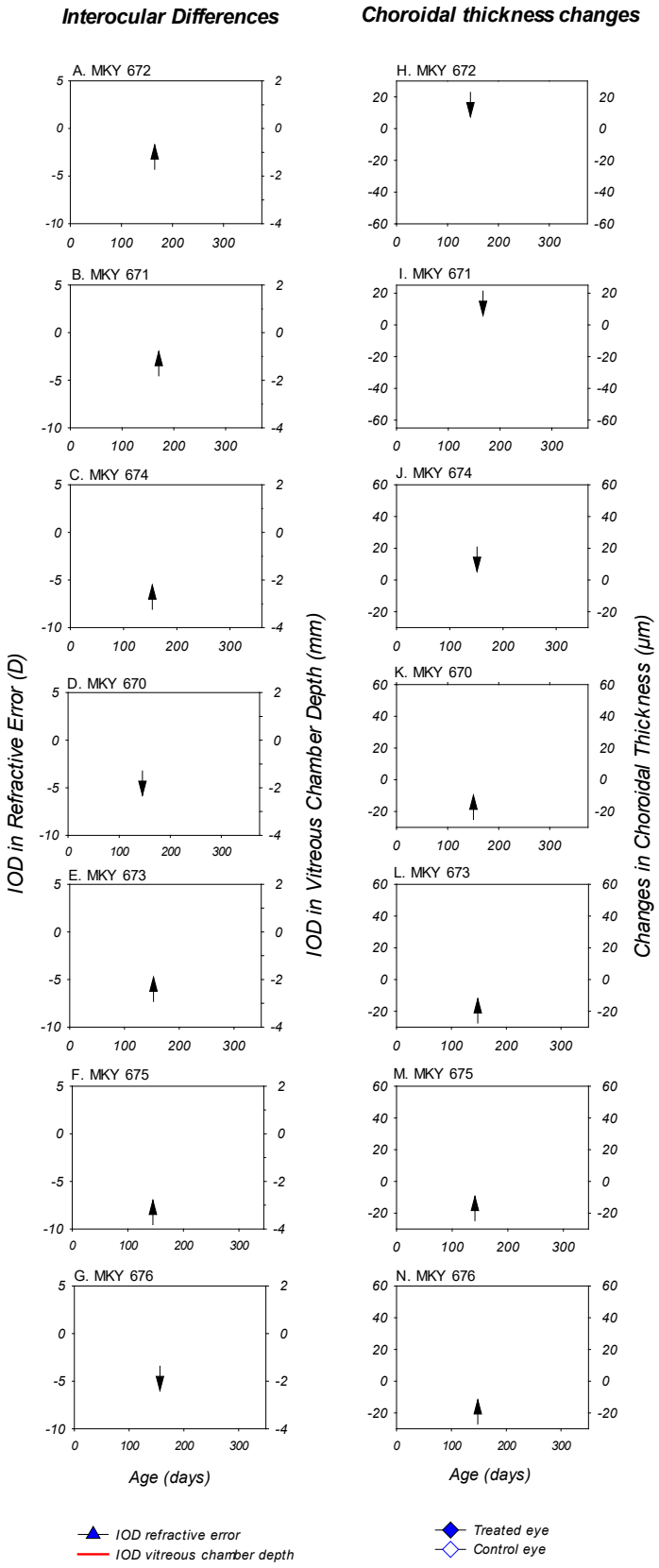
Interocular differences (IOD; treated eye – control eye) in sub-foveal choroidal thickness (red symbols, left ordinates) and refractive error (white symbols, right ordinates) obtained during the dim-light-diffuser periods plotted as a function of age for individual DL-FD subjects. The shaded area represents the 95% confidence range of anisometropias for the NL-FD monkeys during the diffuser-rearing period. The two ordinates are shifted with respect to each other so that the horizontal dashed lines represent zero IODs in refractive error and choroidal thickness.

Figure 3-8. Recovery from FDM



Panels A and B: Anisometropia plotted as a function of age during the recovery period for individual NL-FD (panel A, symbols, n = 10) and DL-FD monkeys (panel B, symbols, n = 5). In panel A, the thin grey lines represent the recovery from FDM for form-deprived monkeys reared with weaker diffusers (Smith III & Hung, 2000) or with intermittent normal vision (Smith III *et al.*, 2002). In panel B, the thin grey lines represent the data from all diffuser-reared monkeys shown in panel A (n = 23). Only monkeys that exhibited at least 1.5 D of myopic anisometropia at the end of the diffuser-treatment period were included in the figure. The one NL-FD monkey that did not recover from FDM is highlighted in yellow (panel A), whereas the three DL-FD monkeys that did not recover are highlighted in blue (panel B). Panel C: Anisometropias obtained at the end of the diffuser treatment period (filled symbols) and at the end of the recovery period (open symbols) plotted for the individual form-deprived animals that are included in panels A and B.

Figure 3-9. Interocular differences in refractive error and vitreous chamber and their relationship with choroidal thickness



Panels A – G (left column): interocular differences (treated eye – fellow eye) in refractive error (blue symbols) and vitreous chamber depth (solid red lines) obtained during the dim-light-rearing period plotted as a function of age for individual DL-FD subjects. Dashed lines: zero anisometropia/interocular difference in refractive error and vitreous chamber depth. Black arrows: ages that correspond to the end of the diffuser treatment. Note the temporal correlation between the IODs in vitreous chamber and refractive error. Panels H – N (right column): changes in choroidal thickness in the treated (filled symbols) and control eyes (open symbols) of the DL-FD monkeys obtained during the recovery period plotted as a function of age. The first symbol in each plot represents data obtained at the age corresponding to the end of the diffuser-rearing period (i.e. black arrows in panels A – G). The choroidal thicknesses are specified relative to the thicknesses obtained at the end of the dim-light-diffuser period. Dashed lines: zero change in choroidal thickness (right column panels).

Chapter 4.

The effects of reduced ambient lighting on lens compensation in infant rhesus monkeys

4.1 Introduction

During early postnatal growth, the eye's optical components (i.e. cornea and crystalline lens) and vitreous chamber develop in a coordinated manner, such that the initial refractive errors in a given eye gradually decrease to near emmetropic levels and the inter-individual differences in refractive error is also reduced. This "emmetropization" process has been observed in humans and most common laboratory species (for a review, see Troilo *et al.*, 2019).

Emmetropization is a vision-dependent process; in particular, the hallmark changes of emmetropization, i.e., the systematic reduction in refractive error and its inter-individual variability, are regulated by visual feedback associated with the eye's effective refractive state. Such a regulatory feedback mechanism was first demonstrated by Schaeffel *et al.* (Schaeffel *et al.*, 1988), who showed that chickens reared with negative- and positive-powered lenses exhibited relative myopic and hyperopic changes, respectively, that eliminated most of the lens-imposed optical errors (i.e., defocus). These compensating refractive changes were achieved by altering the axial elongation rate of the eye's vitreous chamber. Qualitatively similar observations, or "lens compensations", were later reported for mice (Barathi *et al.*, 2008; Jiang *et al.*, 2018; Pardue *et al.*, 2013; Tkatchenko *et al.*, 2010), tree shrews (Metlapally & McBrien, 2008; Siegwart Jr. & Norton, 2010), guinea pigs (Howlett & McFadden, 2009), marmosets

(Troilo *et al.*, 2009; Whatham & Judge, 2001), and macaques (Hung *et al.*, 1995; Smith III & Hung, 1999), suggesting that a widely conserved feedback control mechanism operates to achieve and maintain the optimal refractive state via alterations in vitreous chamber growth.

It has been shown that other elements of visual experience, most notably absolute ambient lighting levels, can influence normal emmetropization and vision-induced alterations in refractive development. For example, elevated ambient lighting levels have been shown to protect a variety of animal species from form-deprivation myopia (FDM) (Siegwart Jr., Ward, and Norton, 2012; Ashby, Ohlendorf, and Schaeffel, 2009; Smith III, Hung, and Huang, 2012; Karouta and Ashby, 2015; Chen *et al.*, 2017). Elevated ambient lighting levels also slowed the reduction in hyperopia associated with normal emmetropization in chicks, resulting in relative hyperopic refractive errors (Cohen *et al.*, 2011;, 2012). With respect to lens-induced changes in refractive development, Ashby *et al.* showed that rearing monocularly defocused chicks under elevated ambient lighting (10,000 lux) slowed the compensation to monocular -7D lenses and accelerated the compensation to monocular +7D lenses in comparison to chickens reared under typical laboratory lighting levels (500 lux), but did not affect the final degree of axial ametropias (Ashby and Schaeffel, 2010). In a later study, Siegwart Jr. *et al.* also found that elevated ambient lighting (16,000 lux) slowed the myopic shifts produced by optically imposed hyperopic defocus in tree shrews and doubled the time required to achieve full compensation (Norton & Siegwart Jr., 2013). Similar results were also reported for guinea pigs reared with monocular -4D lenses (Li *et al.*, 2014). These studies consistently showed that elevated ambient lighting could alter the rate of lens-induced refractive developments in a sign-dependent manner. However, the same elevated ambient lighting paradigms that protected infant rhesus monkeys from FDM (Smith III *et al.*, 2012) did not have a significant effect on either emmetropization in the fellow control eyes or the myopic compensation to negative-lens-induced defocus (Smith III, Hung, Arumugam, & Huang, 2013). The observations in macaque

monkeys suggested that the ambient lighting parameters (i.e. intensity levels and/or length of exposures) required to affect form-deprivation myopia and lens-induced myopia for higher primates might not be identical.

The effects of elevated ambient lighting suggest that lower ambient lighting levels could conversely promote ocular growth and enhance relative myopic development. In this regard, Cohen *et al.*, in the same studies noted above, showed that reducing ambient lighting levels to 50 lux during the normal daily light-on hours accelerated the reductions in hyperopia, producing absolute myopias and increasing the inter-individual variability in refractive error. These observations suggested that dim ambient lighting is a risk factor of myopia (Cohen *et al.*, 2011, 2012). However, rearing infant monkeys with unrestricted vision under reduced ambient lighting (~55 lux) resulted in increases in hyperopia and in the overall variability of refractive errors between subjects (She *et al.*, 2020). In contrast to the results from chickens, none of these monkeys developed myopia. In addition, neither chicks (Ashby *et al.*, 2009) nor infant rhesus monkeys developed more FDM under reduced ambient lighting, suggesting that low lighting intensity, by itself, is not an environmental enhancer of vision-induced myopia (She *et al.*, 2021). Interestingly, reduced ambient lighting interfered with the recovery from FDM and other emmetropizing responses that are normally observed in monkeys previously reared with diffusers (She *et al.*, 2021). The observed failures of emmetropization and recovery from FDM suggest that low ambient lighting levels reduce the operational efficacy of mechanisms that are normally responsible for regulating refractive development, a speculation that has not been directly and longitudinally investigated. The present study was conducted to examine the effects of reduced ambient lighting on the phenomenon of lens compensation in rhesus monkeys.

4.2 **Method**

4.2.1 Animal subjects

The primary subjects were 15 infant rhesus monkeys (Macacca mulatta) acquired at approximately 2 weeks of age. Before the experimental rearing period, subjects were housed in a nursery room that was illuminated on a 12-hour-light/12-hour light-dark cycle (7AM – 7PM) using "white" fluorescent lights (GE Ecolux® Starcoat® T8 F32T8/SP35/ECO, General Electric Co., Boston, MA). The mean ambient lighting intensity in the housing area was 504 ± 168 lux ("normal light" or NL. Correlated color temperature = 3170K). These pre-experiment rearing conditions were identical to those employed in many of our previous studies involving typical laboratory lighting conditions (She *et al.*, 2020).

4.2.2 Experimental strategies

Starting at approximately 24 days of age, the subjects were reared under reduced ambient lighting with the treatment lenses that were randomly assigned to them (see below) until 153 ± 4 and 147 ± 10 days of age for the DL-LIM and DL-LIH groups, respectively.

4.2.2.1 Experimental "dim light"

The reduced ambient lighting levels ("dim light" or DL) employed in the present study were identical to those used in our previous studies (She *et al.*, 2020; She *et al.*, 2021). In brief, an aluminum-deposited, polyester film (Grafix™ Metalized Dura-Lar®, Silver, 0.05mm-thick; Grafix, Maple Heights, Ohio) was closely attached to the fluorescent lighting panels. This strategy maintained the same spectral energy emission profile of the fluorescent lighting, which was identical to that employed to illuminate the pre-experimental housing areas (correlated color temperature = 3170K. She *et al.*, 2020). The average intensity level measured directly under the light panels at the level of the junction between the upper and lower cages was 55 ±

9 lux. The lighting intensity measured in the front of individual cages with the sensor facing horizontally to the outside of the cage ranged between 7-36 lux.

4.2.2.2 Treatment lenses

At the onset of dim-light rearing, the subjects were fitted with goggle-like helmets (Hung *et al.*, 1995; Smith III & Hung, 1999) that held either a -3D (DL-LIM, n = 8) or +3D lens (DL-LIH, n = 7) in front of the treated eye and a zero-power (plano) lens for the fellow control eye. The helmets were custom fitted to each subject and were inspected and adjusted frequently during the daily light-on hours to ensure proper fit and cleanliness.

4.2.3 Control-group subjects

The primary control data were obtained from two groups of age-matched monkeys previously reared under normal ambient lighting levels with either monocular -3D (NL-LIM, n = 16) or +3D treatment lenses (NL-LIH, n = 7). The ambient lighting conditions in their rearing environments were similar to those experienced by the dim-light monkeys prior to the onset of the lens-rearing period. In addition, we also included data from infant monkeys previously reared without visual restrictions under either normal ambient lighting levels ("normal-light controls" or NL-Controls, n = 41) or under identical dim ambient lighting levels ("dim-light controls" or DL-Controls, n = 7) in order to better illustrate the effects of the dim ambient lighting levels on the refractive and ocular component changes produced in response to the imposed defocus. These datasets have been published and discussed previously (Hung *et al.*, 2018, She *et al.*, 2020).

4.2.4 Outcome measures and data collection

Refractive errors and ocular parameters were measured for both eyes of each animal at the onset (baseline) and periodically throughout the lens-rearing period. Refractive errors were measured using retinoscopy by two experienced examiners and were reported as the mean spherical equivalents of the spectacle-plane refractive corrections. Corneal powers were measured using a hand-held keratometer (Alcon Auto-keratometer: Alcon, Inc., St. Louis, MO, USA). The mean spherical equivalent of three independent measurements were reported. The measurement was performed using a corneal topographer (EyeSys, 2000; EyeSys Vision, Inc. Houston, TX, USA.) in some of the baseline measurement sessions when the corneal powers of the infant monkeys were out of the measurement range of the keratometer (> 62 D, about 5% occurrence rate at ages corresponding to the onset of the experiment) (95% limits of inter-instrument agreement = + 0.49 to -0.37 D) (Kee *et al.*, 2002). Ocular axial dimensions were measured with A-scan ultrasonography (OTI-Scan 1000, Ophthalmic Technologies Inc., Downsview, Ontario, Canada) using a 13 MHz transducer. The acoustic parameters of human eyes (cornea and lens: 1641 m/s, aqueous and vitreous: 1532 m/s) (Byrne & Green, 2002) were assumed for the calculation of axial separations, which were reported as the mean of ten independent readings that were obtained along the normal to the corneal apex.

Pupil diameters were measured from videography recordings taken in the front of an animal's respective cage. The detailed method for obtaining pupil diameter and its rationale have been described previously (She *et al.*, 2020).

Sub-foveal choroidal thicknesses were evaluated using spectral-domain, optical coherence tomography (SD-OCT; Spectralis, Heidelberg, Germany) following the methodology described previously (Hung *et al.*, 2018, She *et al.*, 2021). In short, B-scan OCT images along the horizontal meridian that passed through the deepest point of foveal depression were

obtained using the "Enhanced-Depth Imaging" mode of the manufacturer's operating software (Heidelberg Eye Explorer) and were segmented using a customized Matlab program (2019a, MathWorks, Natick, MA, USA). Choroidal thickness, defined as the distance between Bruch's membrane and the outer border of the choroid, was measured perpendicular to the choroidal – retinal pigment epithelium interface. The average choroidal thicknesses of a 300-micron transverse region centered at the deepest point of the foveal depression are reported.

All rearing and experimental procedures followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the University of Houston's Institutional Animal Care and Use Committee.

4.2.5 Statistical methods

Unless otherwise indicated, data are presented as means ± SDs of the mean. Cross-sectional analyses were performed for data obtained at baseline and at the end of the experiment using Student's (for between-group comparisons) and paired t-tests (for between-eye comparisons). For the LIM experiment, analysis of covariance (ANCOVA) was employed to examine the potential main effects of dim light due to a small, but significant, age-difference between the DL-IIM and NL-LIM monkeys at the end of the experiment. When parametric tests could not be applied, a Mann-Whitney rank-sum test (for between-group comparison) or a Wilcoxon signed-rank test (for between-eye comparisons) was employed.

For longitudinal data, mixed-effect model analyses were employed to compare the time-courses for refractive and corneal power development. Specifically, data were fitted as a 2^{nd} order polynomial function of the length of the lens-rearing period to reflect the curvilinear nature of age-related changes in refractive error and corneal power. The syntax was specified in a way that dim-light effects on the rates of development in the early and late stages of the

experiment were represented by the reported linear and quadratic effect coefficients, respectively. This configuration is useful in identifying differences in rates of refractive development at different stages of the experiment (see section 4.3.3 and related discussion). Based on the mixed-effect method, we also examined whether there was a significant dim-light effect on the variability in refractive development using likelihood-ratio tests (S Rabe-Hesketh & Skrondal, 2012).

Pearson correlation and linear regression were employed to examine the relationship between refractive error and axial elongation. To examine the axial nature of refractive error specifically, we used the vitreous chamber to corneal radius ratio (VC/CR ratio) to represent ocular axial dimension in these analyses. This unitless metric accounts for part of the potential influence of inter-individual differences in corneal power on ocular growth and significantly reduces the noise in correlational and regression analyses (She *et al.*, 2020, Smith *et al.*, 2020).

All statistical analyses were performed using STATA (MP14, STATACORP, College Station, TX, USA) at a significance level of 0.05.

4.3 **Results**

4.3.1 Baseline data

As summarized in Table 4-1, there were no statistically significant baseline differences in refraction or any ocular parameters between the DL- and the corresponding NL-groups. In addition, the refractive errors and ocular parameters, except for lens thickness for the NL-LIH group, were binocularly symmetrical in all groups (paired t-test, $p > 0.05$). All refractive error and ocular parameters in the lens-reared animals were also within the mean ± 2 SD range for NL-Control values (Hung *et al.*, 2018).

4.3.2 Pupil diameters

Pupil diameters that are representative of the early and late lens-rearing periods were obtained from the DL-LIM and DL-LIH monkeys, respectively. At 36 ± 7 days of age, the pupil diameters were 5.13 ± 0.54 mm in the treated eyes and 5.15 ± 0.52 mm in the control eyes of the DL-LIM monkeys, both of which were similar to those of DL-Controls in the early stage of dim-light rearing (4.98 ± 0.62 mm and 4.96 ± 0.74 mm for the right and left eyes, respectively), but larger than those obtained from normal-light-reared monkeys (3.58 ± 0.43 mm, $p < 0.001$; Hung *et al.*, 2018). At 101 ± 9 days of age, the pupil diameters of the DL-LIH monkeys were 5.11 ± 0.24 mm and 5.27 ± 0.18 mm for the treated and control eyes, respectively, which were also larger than those obtained from normal-light-reared monkeys at older ages (132 ± 5 days of age; 3.90 ± 0.45 mm and 3.96 ± 0.45 mm for the right and left eyes, respectively; $p < 0.001$). Since pupil diameter increases with age in both normal-light-reared monkeys (Hung *et al.*, 2018) and our DL-controls (She *et al.*, 2020), it appears that the pupil diameters of our DL-subjects remained larger than the average, age-matched, normal-light-reared monkeys throughout the lens-rearing period.

4.3.3 Compensation to negative lenses in dim light

Dim light reduced the probability of compensation to negative lenses. Four of the DL-LIM monkeys did not develop obvious myopic anisometropias (Figure 4-1A – D); the refractive changes in the two eyes of these monkeys varied from small reductions in hyperopia (Figure 4-1A and B) to substantial binocular and symmetric myopic shifts (Figure 4-1C) and absolute myopias (Figure 4-1D). For the remainder of the DL-LIM subjects, myopic anisometropias (relative myopia in the treated eye in comparison to the fellow control eye) developed during the experiment, but these lens-induced anisometropias were not always sustained (Figure 4-1F).

Due to the fact that many DL-LIM subjects failed to develop or maintain myopic anisometropia, the average degree of anisometropic compensation to monocular hyperopic defocus was significantly reduced. As illustrated in Figure 4-2A, all but one of the NL-LIM subjects successfully developed and maintained lens-induced myopic anisometropias. For the DL-LIM monkeys, however, the development of anisometropia was more variable (Figure 4-2B) and its pattern significantly differed from that observed in the NL-LIM monkeys ($z = 3.13$, $p < 0.01$). At the end of the lens-rearing period, the average degree of myopic anisometropia was significantly smaller in DL-LIM group than in the NL-LIM group (DL- vs. NL-LIM: -0.63 ± 0.77D vs. -2.11 ± 1.10D, $t(22) = -3.41$, $p < 0.01$).

In dim light, negative-lens-induced refractive changes were more variable than in normal light, and their magnitude exceeded that normally associated with successful compensation. Figure 4-3 illustrates the longitudinal changes in refractive error for the DL- and NL-LIM monkeys. On average, both DL- and NL-LIM monkeys exhibited reductions in hyperopia in their treated eyes, of which the initial rates were not statistically different (Figure 4-3A). As the experiment continued, the course of treated-eye refractive development started to differ: whereas the reductions observed in the NL-LIM monkeys decelerated after ~70 days of age, there were no obvious signs of slowing for the DL-LIM monkeys until the later stages of the lens-rearing period ($z = -2.17$, $p = 0.03$ for the quadratic component). On the other hand, the control eyes of DL-LIM monkeys showed significantly greater reductions in hyperopia than those observed in the NL-LIM group soon after the onset of lens-rearing ($z = 3.49$, $p < 0.01$ for the linear component). The rate of the reductions in control eye hyperopia in the DL-LIM group became greater than that observed in NL-LIM (Figure 4-3A, open symbols) and in the NL-Controls (Figure 4-3A, shaded area), but they did not exceed the rate of change observed in the contralateral treated eyes of the DL-LIM animals (Figure 4-3A, filled red symbols). For both the treated- and fellow-control eyes, refractive development (likelihood-ratio test, $p < 0.05$)

and the average refractive changes at the end of the treatment period (DL- vs. NL-LIM, treated eyes: -4.01 ± 3.28D vs. -2.80 ± 1.38D, $z = 0.85$, $p = 0.39$; control eyes: -3.38 ± 3.23D vs. -0.74 ± 1.15D, $z = 1.69$, $p = 0.09$; Figure 4-3B) were significantly more variable in the DL-LIM group than those observed in the NL-LIM group. As a consequence, the differences in average refractive error between the DL-LIM and NL-LIM groups were not statistically significant at the end of the lens-rearing period (Table 4-2); however, both eyes of the DL-LIM monkeys were more myopic than those of the DL-Controls (+3.66 ± 1.75D and +3.70 ± 1.36D for the right and left eyes, respectively; $p < 0.05$) (Figure 4-3C).

4.3.4 Compensation to positive lenses in dim light

The development of LIH was severely curtailed by the dim-light rearing regimen. Figure 4-4 showed that only two DL-LIH monkeys developed obvious hyperopic anisometropias (Figure 4-4A and B). Three subjects did not develop (Figure 4-4C and 4E) or failed to maintain obvious hyperopic anisometropias throughout the experiment (Figure 4-4D). Most remarkably, two monkeys developed myopic anisometropias during lens-rearing (Figure 4-4F and G), which exacerbated, rather than reduced, the lens-imposed myopic defocus. In addition, for all DL-LIH subjects, the control eyes also showed attenuated or relatively normal reductions in hyperopia during the experiment.

Due to the absence of expected and appropriate treated-eye refractive changes, the normally consistent development of compensating hyperopic anisometropias was disrupted in the DL-LIH monkeys. Figure 4-5 illustrates the longitudinal changes in anisometropia for NL- and DL-LIH monkeys. In comparison to the NL-LIH monkeys, DL-LIH monkeys showed substantially higher inter-individual variability in the degree and direction of anisometropic changes, thus the patterns of anisometropia-development were significantly different ($z = -2.13$, $p = 0.03$). At the end of the lens-rearing period, the DL-LIH monkeys were, on average, isometropic (-0.18

± 1.93D vs. +1.71 ± 0.39D in the NL-LIH group; Mann-Whitney test, $z = 2.56$, $p = 0.01$) and the between-subject variability in anisometropia was significantly greater than that in the NL-LIH monkeys (variance ratio test, $F = 0.04$, $p < 0.01$).

Similar to that observed for LIM, the course of refractive development in response to monocular positive-lens wear was more variable in dim light (Figure 4-4; likelihood-ratio test, $\chi = 11.19$, $p = 0.004$). Figure 4-6A illustrates the longitudinal changes in mean refractive error for the NL-LIH and DL-LIH monkey. For subjects reared under normal light, the monocular +3D lenses largely eliminated the normal age-related reductions in hyperopia in the treated eyes (filled black symbols), resulting in smaller overall changes in refractive error in comparison to their fellow control eyes (Figure 4-6B) and consistent compensating hyperopic anisometropias at the end of the lens-rearing period (Figure 4-6C). For the DL-LIH monkeys, the treated eyes exhibited significant myopic shifts in comparison to the NL-LIH monkeys ($z = -2.48$, $p = 0.01$ for the linear component); the time-course of these changes was similar to that observed in their fellow-control eyes (Figure 4-6A, red symbols), both of which were close to the upper 95% limit observed in the NL-controls. As a consequence, the typical developmental pattern of compensating anisometropia was not observed in the DL-LIH monkeys. As illustrated in Figure 4-6B, at the end of the lens-rearing period, the treated eyes of the DL-LIH monkeys exhibited relative myopic changes that were comparable to those in their fellow control eyes (-0.92 ± 2.00 D and -0.67 ± 1.33 D for treated and control eyes, respectively); the refractive errors in both the treated and fellow control eyes of the DL-LIH monkeys did not differ significantly from those in the NL-LIH subjects and the DL-Controls.

4.3.5 Ocular components and the axial nature of the observed refractive errors

We did not observe any significant differences in corneal powers, lens thicknesses, or anterior chamber depths that could account for the observed alterations in refractive error. For corneal

powers specifically, the time course of corneal power development and the corneal powers obtained at the end of the experiment were both similar in the DL- and the corresponding NL-groups (Table 4-2 and Table 4-3), which is in agreement with our previous dim light investigations (She *et al.*, 2020; She *et al.*, 2021). Except for the DL-LIM group, which exhibited a significant, but negligible, interocular difference in lens thickness at the end of the lens-rearing period (Table 4-2), there were no significant between-eye differences in corneal power, anterior chamber depth, or lens thickness.

Differences in vitreous chamber elongation were associated with the observed variability in refractive error and the development of compensating changes in response to imposed defocus. At the end of the lens-rearing period, there was a significant correlation between refractive error and the vitreous chamber to corneal radius ratio (VC/CR ratio) ($r = -0.89$, $p < 0.01$, Figure 4-7A). A linear regression analysis showed that variations in the VC/CR ratio accounted for 80% of the variability in refractive error ($r^2 = 0.8$, $p < 0.01$). In addition, as shown in Table 4-2 and Table 4-3, the less hyperopic/more myopic eyes in both DL-groups also exhibited greater vitreous chamber depths at the end of the experiment. These interocular differences in vitreous chamber depth were smaller in magnitude in the DL-groups in comparison to those observed in the corresponding NL-groups (DL- vs. NL-LIM: +0.21 ± 0.25mm vs. +0.38 ± 0.24mm, $t(22) = -1.61$, $p = 0.12$; DL- vs. NL-LIH: +0.09 ± 0.47mm, -0.33 ± 0.08mm, $t(12) = 2.36$, $p = 0.04$). Finally, the interocular differences in vitreous chamber depth observed at the end of the experiment were inversely correlated with the interocular differences in refractive error ($r = -0.92$, $p < 0.01$; Figure 4-7B). Thus, the refractive alterations observed in the present study showed the same axial nature as those observed under normal ambient lighting.

4.3.6 Choroidal thickness changes

The observed failures to respond to imposed defocus were associated with an absence of the relative choroidal thickness changes that are normally induced by defocus. To examine the relationship between choroidal thickness change and refractive development for our DL-LIM monkeys, we segregated this group based on whether negative-lens compensation had occurred and then compared the longitudinal changes in choroidal thickness in these two subgroups. In comparison to the subgroup that did not show obvious compensating changes (the "isometropic subgroup", Figure 4-8B), in which the choroidal thickness changes were similar in the treated and control eyes (Figure 4-8D), the subgroup that developed obvious compensating refractive changes (the "anisometropic subgroup", Figure 4-8A) exhibited initial interocular differences in choroidal thickness that were in the appropriate direction in relation to the nature of the imposed defocus, i.e. relative choroidal thickening in the eyes that were more hyperopic/less myopic (Figure 4-8C). These interocular differences were due primarily to the abnormal age-related changes in the treated eye's choroidal thickness. Specifically, the increases in treated-eye choroidal thickness in the anisometropic subgroup were much smaller in magnitude in comparison to those observed in their control eyes and those in the treated eyes of the isometropic subgroup. In contrast, the isometropic subgroups did not show any systematic interocular differences in choroidal thickness at any point throughout the experiment. At the end of the experiment, there were no significant interocular differences in choroidal thickness in either subgroup (treated vs. control eye, anisometropic subgroup: 40.37 ± 7.94 vs. 48.58 ± 14.3 μm, $t(3) = -0.87$, $p = 0.45$; isometropic subgroup:, 19.01 ± 9.89 vs., 21.48 ± 9.22 μm, $t(3) = -0.16$, $p = 0.88$).

Similar to the associations observed in the DL-LIM subjects, interocular differences in choroidal thickness change that were in the appropriate direction to compensate for the imposed

defocus (i.e. relative thickening in the treated eye, illustrated as positive interocular differences) were observed early in the experiment in the subjects that developed obvious degrees of hyperopic anisometropia (monkeys 742, 727 and 731, Figure 4-9A – C). For the subjects that remained relatively isometropic (monkey 732, Figure 4-9D) or developed myopic anisometropia (monkeys 730, 728 and 726, Figure 4-9E – G), the expected relative increases in choroidal thickness in response to the imposed myopic were not observed in their treated eyes. On the contrary, there was a trend for the treated-eye choroids be thinner in comparison to their fellow control eyes.

4.4 **Discussion**

We found that dim light rearing reduced the probability of refractive changes that compensate for lens-imposed defocus, increased the variability in refractive development between subjects, and reduced the average degree of compensating anisometropias. The failure to exhibit compensating refractive changes was associated with an absence of the relative choroidal thickness changes that are normally induced by defocus under normal ambient lighting. The inter-individual variability in refractive development and the inconsistent compensation that was observed in both groups of DL monkeys were axial in nature, like that normally associated with lens compensation under normal ambient lighting.

4.4.1 Effects of dim light on corneal power

We did not find any significant dim-light effects on corneal power development. This observation is in agreement with the findings from our previous investigations of emmetropization (She *et al.*, 2020) and FDM in monkeys that were reared under similar dim ambient lighting (She *et al.*, 2021). Together with the observations under elevated ambient

lighting levels (Smith III *et al.*, 2012, 2013), our data indicate that corneal power development in infant rhesus monkeys is largely unaffected by different ambient lighting levels. In contrast, both elevated and reduced lighting levels have been found to affect corneal power development in chicks (Cohen *et al.*, 2011;, 2012). In addition, negative-lens-imposed defocus has been reported to cause corneal steepening in mice reared under ambient lighting levels that were similar to the dim lighting levels employed in the present study (Landis *et al.*, 2021). It appears that the biometrical nature of the refractive responses to light-level manipulations or the interaction between imposed defocus and reduced lighting levels might not be identical in avian and rodent species versus non-human primates.

4.4.2 Pupil dilation and its potential influence

The effects of ambient illumination levels are presumably mediated by vision-related mechanisms. In this respect, a more relevant measure for the environmental stimulus would be the illumination level on the retina (i.e. retinal illumination level. Thibos *et al.*, 2018; Troland, 1917), which requires that alterations in ambient illumination should be scaled by pupil size. In our experiment, the relative pupil dilation observed in DL-subjects (1.0 – 1.5mm larger in average diameter than NL-subjects, or 32% - 44% in percentage increases) was similar to that observed in the DL-Controls (She *et al.*, 2020), resulting in 74% - 107% increases in pupil area in comparison to those in NL-subjects. As a numerical example of how this pupil size difference affects retinal illumination, an area on the white-painted walls (the brightest of all common visual targets in our dim-light housing) that subtends a 1° visual angle produces 1.51×10^{-4} photopic trolands of retinal illumination through the relatively dilated (5.14mm) pupil in a DL subject that is viewing along the normal to the wall at cage-level. In comparison to that the retinal illumination produced by dim light through a non-dilated pupil (3.58mm, 7.32×10^{-5} photopic trolands), the larger pupils in the DL monkeys nearly doubled the effective retinal

illumination. However, the resulting retinal illumination remained ~6 times lower than that in "normal" light (9.76×10^{-4} photopic trolands). This example showed that the larger pupil size observed among DL-subjects could improve retinal illumination and partially offset the reductions in ambient illumination; however, due to its unidirectional nature, this effect did not appear adequate to explain the high inter-individual variability, the absence of light-level-associated systematic refractive error alterations, and particularly the difference between DL-lens-reared subjects and the DL-Controls.

Although pupil dilation might also affect the efficacy of our lens treatment through alterations in depth-of-field (DOF), the increases in pupil size observed among DL subjects produced only a small difference in DOF (estimated magnitude based on axial dimensions and visual acuity = +0.25D at ages that correspond to the onset of lens-rearing; Green *et al.*, 1980; She *et al.*, 2020) in relation to the powers of the treatment lenses (±3D). This DOF difference was also small in comparison to the changes in absolute refractive error that are normally associated with lens compensation, particularly those observed in the DL-subjects that developed obvious myopic changes. In this respect, it seems unlikely that alterations in the eye's DOF associated with larger pupil diameters, by itself, had a significant influence on an eye's effective refractive state and the magnitude of refractive changes induced by the imposed defocus.

The larger pupil diameters in our DL monkeys could have increased the magnitude of their eyes' higher-order monochromatic aberrations (HOAs), which could potentially influence emmetropization and the compensation to imposed defocus. For example, a greater magnitude of HOAs at the onset of the experiment might reduce the efficacy of emmetropization (see the next section) through retinal image degradation. In addition, greater magnitudes of HOAs could also result in further increases in DOF (Charman, 2005). For animals that developed myopic

changes in the early stages of lens-rearing, larger pupil size might have also exaggerated the influence of myopia-related increase in HOAs (Coletta *et al.*, 2003, 2010; García De La Cera *et al.*, 2006; Kisilak *et al.*, 2006; Ramamirtham *et al.*, 2007; Tian & Wildsoet, 2006) on retinal image quality, which might in-turn enhance the myopic changes. It should be noted that other factors, including changes in the shapes and alignment of the cornea and lens, as well as changes in axial dimensions, might also affect the nature (and probably the magnitude) of HOAs. Therefore, the extent to which pupil-size-related alterations in HOAs affected the refractive development of our DL subjects remains unclear. Given that some eyes with large experimental myopia and presumably increased HOAs recovered when treatment lenses were removed (Qiao-Grider *et al.*, 2004; She *et al.*, 2021), we speculate that any contribution of HOAs, albeit probable, was relatively small.

4.4.3 Dim-light effects on lens-induced refractive compensation

We found that, in comparison to typical laboratory lighting levels, dim ambient lighting significantly increased the between subject variability in refractive development. Similar observations have been reported for infant rhesus monkeys reared with unrestricted vision (She *et al.*, 2020) and, in an analogous manner, for monkeys undergoing recovery from FDM (She *et al.*, 2021). It has also been reported that unrestricted refractive development is more variable in chickens reared under low ambient lighting, whereas under typical or elevated ambient lighting levels there was less inter-individual variability around the eventual target refractive error (Cohen *et al.*, 2011, 2012).

In comparison to that observed under typical laboratory lighting levels (Hung *et al.*, 1995; Smith III & Hung, 1999), dim light reduced the likelihood of systematic compensating changes in refractive error in response to both imposed hyperopic and myopic defocus. This effect is illustrated in Figure 4-10, which plots the effective refractive error, defined as the through-the-

lens refractive error for treated eyes of the lens-reared monkeys and the uncorrected refractive errors for controls and monkeys undergoing recovery from FDM, obtained at the onset and end of the relevant observation periods for individual monkeys reared under different lighting conditions. Under typical ambient lighting, eyes with a large range of initial effective refractive errors consistently emmetropized towards the target range of refractive errors for normal control monkeys (mean ± 2SD range of the NL-controls, Figure 4-10A, solid and dash lines). In contrast, many of our DL subjects either showed little change in effective refractive error or showed changes that were not in the appropriate directions (Figure 4-10B), despite the fact that their initial effective refractive errors were within the range of refractive errors that supported the expected responses in normal light. In particular, in dim light, eyes that presented with a significant dioptric stimulus for emmetropization (i.e., high degrees of hyperopia) often failed to exhibit emmetropization-associated reductions in hyperopia, whereas eyes that presented with optical "stop" signals (relative or absolute myopic defocus) frequently showed no compensating changes or exhibited inappropriate myopic changes. As a result, dim-light-rearing virtually eliminated the hallmark patterns in refractive development that are associated with normal active visual regulation (i.e., emmetropization, recovery from FDM, and lens-compensation), indicating that the efficacy of the feedback control mechanisms that regulates refractive development is reduced under dim lighting.

Do the myopias observed in the treated eyes of some of our dim-light subjects, especially those in the DL-LIM monkeys, reflect a systematic myopiagenic effect? In the present study, we did not find evidence for an acceleration or deceleration in lens compensation analogous to the observations in chicks reared with imposed defocus under elevated ambient lighting (Ashby *et al.*, 2012). In addition, neither the present investigation, nor our previous study on emmetropization, found signs of consistent, unidirectional increases in the age-related reductions in hyperopia that were analogous to those observed in dim-light-reared chickens

(Cohen *et al.*, 2011, 2012). Due to the absence of systematic alterations in the endpoint ametropia and the presence of large inter-individual variability, the substantial myopic changes observed in some of the lens-treated eyes are probably best explained by the reduced efficacy in the emmetropization process noted above. It is possible that the myopias developed because the dim light reduced the ability of regulatory mechanisms to encode or respond to optical signals that would normally reduce axial elongation. For example, for some DL-LIH monkeys, the age-related, "intrinsic" ocular growth could have dominated refractive development in the absence of optical "stop" signals that are normally associated with positive lens-wear (in a manner somewhat similar to that associated with FDM). For the DL-LIM monkeys, individual failures in detecting or processing the optical "stop" signals in their visual environment might have contributed to the sustained myopic changes observed in the later stage of the experiment. If this scenario is correct, the reduced responsiveness to absolute (She *et al.*, 2021) and relative myopic defocus under dim ambient light could have significant consequence with respect to the development of myopia.

Dim ambient lighting increased the variability in refractive development in the control eyes of both DL-groups, which appeared to agree with a reduction in the efficacy of visual regulation. In addition, dim light also caused obvious myopic changes in the control eyes of the DL-LIM monkeys that developed myopic change in their treated eyes, a phenomenon that was not observed in the DL-controls nor in the control eyes of the NL-LIM monkeys. However, similar changes have been observed in the control eyes of form-deprived monkeys reared under dim light that developed myopia in their treated eyes (She *et al.*, 2021). This between-study agreement suggests that the combination of dim light and vision-induced myopia in the treated eye can influence control eye refractive development (She *et al.*, 2021). In relation to our speculations above, a reduction in the efficacy of the vision-dependent growth regulating mechanisms in the control eyes might increase the eyes' susceptibility to the interocular factors

associated with vision-induced myopia, i.e. a “contralateral eye influence” that causes myopic changes (Bradley *et al.*, 1999; Raviola & Wiesel, 1985; Schmid & Wildsoet, 1996; Smith *et al.*, 1987; Smith III & Hung, 2000; Wildsoet & Wallman, 1995).

Based on the above reasonings, we believe that reduced ambient lighting does not produce a systematic myopiagenic effect per se; instead, it reduces the efficacy of visual regulatory mechanisms that normally operate to optimize the existing refractive errors and to prevent myopia. For our subjects, this effect, particularly the absence of “stop” responses to relative myopic defocus, resulted in the increased occurrence of myopia.

4.4.4 Choroidal thickness changes and their implications

Vision-induced choroidal thickness changes in response to optical defocus (Wallman *et al.*, 1995) are consistently observed in normal-light-reared animals (Wallman *et al.*, 1995; Howlett and McFadden, 2006; Siegwart Jr. and Norton, 1998; Hung, Wallman, and Smith III, 2000; Troilo, Nickla, and Wildsoet, 2000a). In the present study, the successful development of compensating anisometropias was associated with relative choroidal thickness changes that were in the appropriate direction for the sign of lens-imposed defocus. Similarly, in a previous study, sign-appropriate changes in choroidal thickness were only observed in monkeys successfully recovering from FDM in dim ambient lighting (She *et al.*, 2020), which suggests that the absence of sign-appropriate choroidal thickness changes in the dim-light-reared monkeys that did not recover from FDM reflected a failure to respond to the existing defocus signals (She *et al.*, 2021). In this respect, the observations of the present study also suggest that dim ambient lighting reduces the ability of the emmetropization process to detect and/or respond to the optically imposed interocular differences in refractive error, resulting in an absence of consistent lens-compensating responses. Thus, our choroidal thickness observations

indicate that low intensity ambient lighting reduces the efficacy of retinal mechanisms responsible for emmetropization.

4.4.5 Possible explanations for the reduced efficacy of visual regulation

It is suggested that the visual cues required for emmetropization might be attenuated under dim light (Wallman *et al.*, 1995). However, many parametric retinal image properties that are speculated to provide cues for emmetropization do not seem to change under reduced ambient lighting. For example, our ambient lighting paradigm preserved the relative spectral output profile across the visible spectrum (She *et al.*, 2020), thereby maintaining the potential sign-of-defocus cues associated with the eye's longitudinal chromatic aberration (Gawne & Norton, 2020). In addition, the filters that we used to reduce the ambient lighting levels produced uniform percentage reductions in energy irradiance across the visible spectrum (She *et al.*, 2020; or see Figure 2-1). As a consequence, common luminance contrast statistics (e.g. Weber's contrast and Michelson contrast; see Rucker & Wallman, 2012), and probably the spatial frequency information, would remain unchanged. In this respect, many potential emmetropization cues, defined by common parametric characteristics of image properties, are likely preserved under reduced ambient lighting.

Although the ambient lighting levels employed in our studies remained within the lower range of primate photopic vision, the resulting visual environment was perceptually dim for humans and probably infant monkeys. Thus, it is reasonable to assume that retinal adaptation in response to the reduced ambient lighting took place, and that some of these functional changes might have contributed to the speculated reduced efficacy of the emmetropization process. For example, low ambient lighting levels might increase the coupling of horizontal cell gap junctions and thus their receptive field size through light-level-associated changes in retinal dopamine production (i.e., events that are opposite to those that occur during light

adaptation, see Baldridge, 2001; Dong & McReynolds, 1991; Weiler & Akopian, 1992; Zhang *et al.*, 2011), thereby increasing the responsiveness to photic stimulation at the expense of spatial resolution. Although emmetropization does not necessarily rely on retinal mechanisms that are associated with high spatial resolution (Gawne & Norton, 2020; Rucker & Wallman, 2012; Schmid & Wildsoet, 2004), this example nonetheless demonstrates that some retinal functions (in this case, one that is important for emmetropization) are in a sub-optimal state under dim light. It is possible that dim-light produced the observed refractive effect by altering the activity level of retinal neuromodulators, most notably dopamine (Brainard & Morgan, 1987; Iuvone, Galli, & Neff, 1978), thus altering the functional state of other retinal neurons and/or pathways. Dopamine is a probable candidate molecule in this respect due to its extensive involvement in the modulation of retinal function (Witkovsky & Dearry, 1991). Studies have shown that many retinal pathways that are subject to dopaminergic regulation (Chaffiol *et al.*, 2017; Feigenspan & Bormann, 1994; Mazade *et al.*, 2019; Mazade & Eggers, 2019; Qiao *et al.*, 2016; Wellis & Werblin, 1995) play important roles in refractive development and in experimental myopia (for a review, see Zhou *et al.*, 2017). If the dopaminergic system was indeed involved in the observed dim-light effects, the inter-individual variability in refraction might be attributed to the individual differences in the retinal dopaminergic system and to the inter-individual differences in light-level-associated alterations in retinal dopaminergic activity.

4.4.6 Summary and implications

Rearing infant monkeys under reduced ambient lighting attenuated the compensation to negative lenses and severely disrupted the compensation to positive lenses. These effects came about because the eyes had a lower probability of responding appropriately to the presenting refractive state. In agreement with our previous observations that dim-light-rearing reduced the

probability of successful emmetropization (She *et al.*, 2020) and the recovery from FDM (She *et al.*, 2021, in press), the results of this study suggest that low intensity ambient lighting affects the defocus-driven mechanisms which normally regulate refractive development. In support of this view, we found that failures of lens compensation were associated with the absence of appropriate choroidal thickness changes. This finding was similar to that observed in dim-light-reared monkeys underdoing recovery from FDM, both suggesting that failure to detect and/or process optical signals was responsible for the failure to initiate compensating refractive changes in individual animal.

It should be noted that our animals did not have access to any higher ambient illuminations, a situation that rarely, if ever, occurs in real life (Ostrin, 2017). Given that animals emmetropize normally under ambient illuminations that are at or above typical laboratory lighting levels, any access to higher ambient lighting conditions would allow a period of time for the eyes to correctly detect and respond to the eyes' refractive state, whereby overcoming, at least potentially, any adverse refractive consequences. In this respect, our long and consistent experimental paradigm might have exaggerated the refractive effect of low intensity ambient lighting on refractive development. Despite this limitation, our study clearly showed that extended exposure to low intensity ambient lighting could impair normal refractive development and might be myopiagenic for some young animals that are undergoing emmetropization. On the other hand, our findings suggest that the typical laboratory lighting levels that are commonly employed in animal experiments are sufficient to ensure normal emmetropization and to protect young animals from developing refractive anomalies such as those observed in the present study. In comparison to the elevated ambient lighting levels that have been shown to protect animals from FDM (2,500 – 40,000 lux, Chen *et al.*, 2017; Karouta & Ashby, 2015; Siegwart Jr. *et al.*, 2012; Smith III *et al.*, 2012), the relative increase in ambient illuminance that appears to be protective against dim-light-associated refractive anomalies

observed in this and our previous studies are clearly much smaller and can be more practically obtained. In this respect, a recent clinical study showed that elevating classroom desk/blackboard illumination levels from under 100 lux to just ~400 - 500 lux significantly reduced the onset of myopia and slowed axial myopic progression in school-age children (Hua *et al.*, 2015). The anti-myopia effect associated with this modest improvement in classroom illumination might be associated, at least in part, to the "protective effect" of "typical" ambient lighting suggested by our dim-light observations.

Acknowledgement

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Tables and Figures

Table 4-1. Baseline data

	NL-LIM (24 ± 2 days)		DL-LIM (23 ± 2 days)		NL-LIH (24 ± 2 days)		DL-LIH (25 ± 2 days)	
	Treated eye	Control eye	Treated eye	Control eye	Treated eye	Control eye	Treated eye	Control eye
Refractive error (D)	+3.64 ± 1.05	+3.69 ± 1.18	+4.48 ± 0.99	+4.47 ± 0.95	+4.61 ± 1.2	+4.66 ± 1.26	+3.47 ± 1.61	+3.40 ± 1.61
Corneal power (D)	61.19 ± 1.09	61.11 ± 1.16	60.18 ± 1.26	60.12 ± 1.30	62.18 ± 1.86	62.02 ± 1.59	61.39 ± 1.27	61.28 ± 1.42
Anterior chamber depth (mm)	2.49 ± 0.14	2.51 ± 0.15	2.48 ± 0.13	2.49 ± 0.13	2.43 ± 0.12 *	2.59 ± 0.18 *	2.43 ± 0.11	2.44 ± 0.10
Lens Thickness (mm)	3.67 ±0.17	3.67 ± 0.15	3.68 ± 0.33	3.39 ± 0.34	3.62 ± 0.22	3.60 ± 0.18	3.73 ± 0.11	3.74 ± 0.12
Vitreous chamber depth (mm)	8.62 ± 0.36	8.63 ± 0.35	8.62 ± 0.24	8.64 ± 0.23	8.41 ± 0.33	8.38 ± 0.33	8.52 ± 0.30	8.51 ± 0.30

*Significant between-eye difference

Baseline refractive error and ocular parameters for the LIM and LIH experiments. The significant difference in anterior chamber depth observed in the NL-LIH group likely reflects measurement noise.

Table 4-2. Refraction and ocular parameters at the end of the experiment for LIM monkeys

	NL-LIM **(144 ± 6 days)** *			DL-LIM **(153 ± 4 days)** *				
	Treated eye	Control eye	Between-eye comparison	Treated eye	Compare to NL-LIM	Control eye	Compare to NL-LIM	Between-eye comparison
Refractive error (D)	+0.84 ±1.89	+2.95 ± 1.37	**t (15) = -7.67, p < 0.01**	+ 0.47 ± 3.00	F (1, 21) = 0.33, p = 0.57	+1.09 ± 2.91	z = 1.35, p = 0.18	**t (7) = -2.31, p = 0.06**
Corneal power (D)	55.13 ± 1.49	55.01 ± 1.59	t (15) = 0.99, p = 0.34	54.39 ± 1.4	F (1, 21) = 2.75, p = 0.11	54.25 ± 1.61	F (1, 21) = 2.28, p = 0.15	t (7)= 0.69, p = 0.51
Anterior chamber depth (mm)	3.11 ± 0.15	3.10 ± 0.13	t (15) = 1.03, p = 0.32	3.03 ± 0.11	F (1, 21) = 0.25, p = 0.62	3.06 ± 1.21	F (1, 21) = 0.04, p = 0.85	t (7) = -2.02, p = 0.08
Lens Thickness (mm)	3.64 ± 0.12	3.65 ± 0.13	t (15) = -0.58, p = 0.57	3.77 ± 0.22	F (1, 21) = 1.28, p = 0.27	3.73 ± 0.22	F (1, 21) = 0.98, p = 0.33	**t (7) = 2.50, p = 0.04**
Vitreous chamber depth (mm)	10.40 ± 0.55	10.01 ± 0.49	**t (15) = 6.41, p < 0.01**	10.64 ± 0.79	F (1, 21) = 1.25, p = 0.28	10.42 ± 0.90	z = -1.44, p = 0.15	**t (7) = 2.46, p = 0.04**

*. Significant between-group difference.

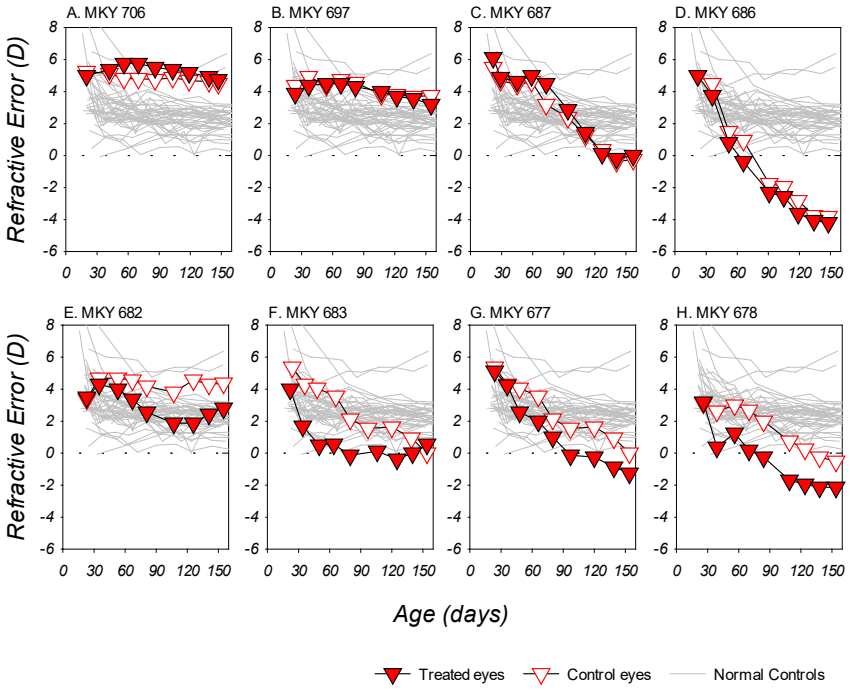
Refractive errors and ocular parameters obtained at the end of the lens-rearing period for NL- and DL-LIM monkeys. Statistically significant between-eye difference in refraction and ocular parameters were highlighted in bold. For the ANCOVA tests reported in the table, there was no evidence to reject the equal-slope assumption (i.e. there were no significant interactions between ambient lighting levels and length of treatment). The small, but significant, between-eye difference in lens thickness in the DL-LIM group likely reflected measurement noise.

Table 4-3. Refraction and ocular parameters at the end of the experiment for LIH monkeys

	NL-LIH (133 ± 14 days)			DL-LIH (147 ± 10 days)				
	Treated eye	Control eye	Between-eye comparison	Treated eye	Compare to NL-LIM	Control eye	Compare to NL-LIM	Between-eye comparison
Refractive error (D)	+4.63 ± 0.91	+2.92 ± 0.89	**t (6) = 11.73, p < 0.01**	+ 2.55 ± 2.43	t (12) = 1.19, p = 0.26	+2.73 ± 1.07	t (12) = 0.36, p = 0.73	t (6) = -0.24, p = 0.82
Corneal power (D)	55.82 ± 1.65	55.61 ± 1.23	t (6) = -1.48, p = 0.19	55.30 ± 1.25	t (12) = -0.33, p = 0.75	55.33 ± 1.21	t (12) = 0.06, p = 0.95	t (6) = -0.16, p = 0.88
Anterior chamber depth (mm)	3.08 ± 0.08	3.05 ± 0.09	**t (6) = 3.67, p = 0.01**	3.07 ± 0.19	t (12) = 0.04, p = 0.97	3.10 ± 0.12	**t (12) = -2.25, p = 0.04**	t (6) = -0.53, p = 0.61
Lens Thickness (mm)	3.55 ± 0.14	3.56 ± 0.15	t (6) = -1.21, p = 0.27	3.68 ± 0.17	t (12) = -0.16, p = 0.88	3.67 ± 0.20	t (12) = 0.41, p = 0.69	t (6) = 2.50, p = 0.81
Vitreous chamber depth (mm)	9.51 ± 0.33	9.85 ± 0.33	**t (6) = -11.34, p < 0.01**	10.07 ± 0.47	t (12) = -2.18, p = 0.050	9.98 ± 0.34	t (12) = -0.03, p = 0.97	t (6) = 0.51, p = 0.63

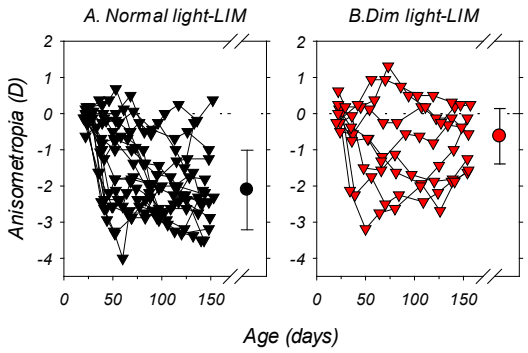
Refractive errors and ocular parameters obtained at the end of the lens-rearing period for NL- and DL-LIH monkeys. Statistical significance is highlighted in bold.

Figure 4-1. Refractive development for individual DL-LIM monkeys



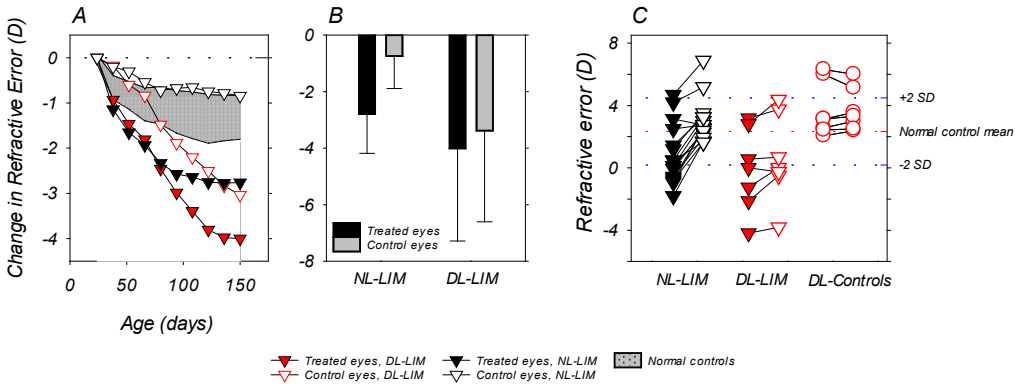
Refractive error plotted as a function of age for individual DL-LIM monkeys. The filled and open symbols represent data from the treated and control eyes, respectively. Data for the right eyes of the normal control monkeys were plotted as thin grey lines.

Figure 4-2. The development of compensating myopic anisometropias



Anisometropia (treated eye ametropia – control eye ametropia) plotted as a function of age for individual animals in the NL-LIM (panel A) and DL-LIM groups (panel B). The circular symbol with error bars in each panel represents the mean (±SD) anisometropia obtained at the end of the lens-rearing period.

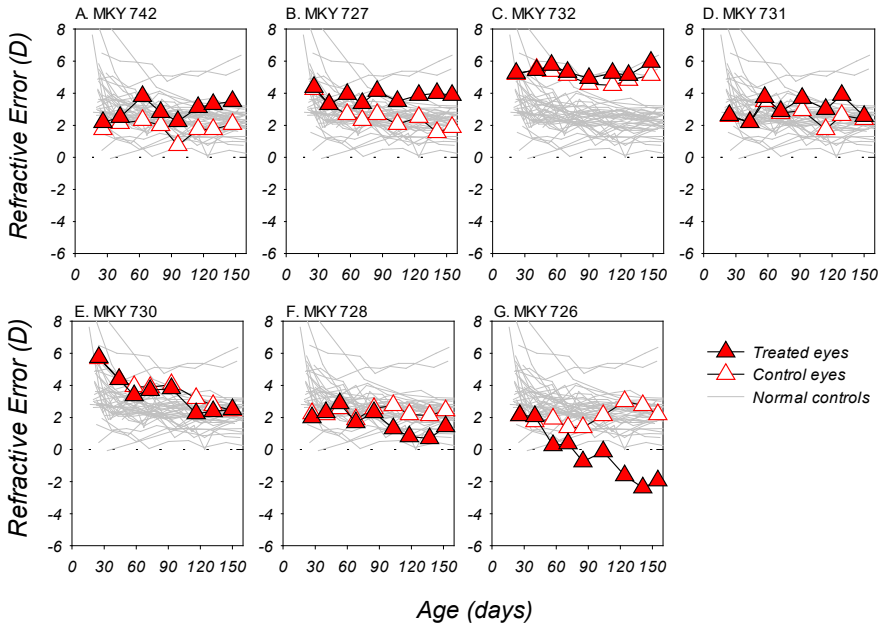
Figure 4-3. Refractive changes and end-of-treatment refractive errors for the LIM monkeys



Panel A. Mean changes in refractive error plotted as a function of age for DL-LIM and NL-LIM monkeys. To calculate the mean changes, the refractive data obtained at each measurement were linearly interpolated to the closest age-point in an equally spaced age sequence (range between 24-150 days, inclusive) and averaged. Both DL- and NL-LIM groups exhibited similar rapid initial reductions in hyperopia. However, the DL-LIM group maintained

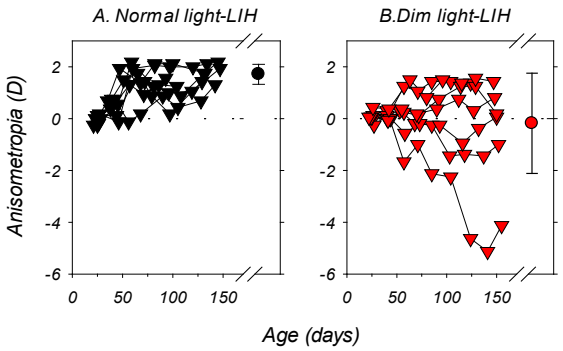
its initial rate of hyperopia reduction for a longer period than the NL-LIM group and developed greater relative myopic changes. The control eyes of the DL-LIM monkeys also exhibited large myopic shifts in comparison to NL-LIM subjects and normal controls. Filled symbols: treated eye; open symbols: control eyes; red symbols: DL-subjects; black symbols: NL-subjects. Panel B. Mean changes in refractive error obtained on the last day of the lens-rearing period. Panel C. Refractive errors obtained at the end of the lens-rearing period for the treated (filled symbols) and control eyes of the lens-treated monkeys and at ages corresponding to the end of lens-rearing period for dim-light control monkeys.

Figure 4-4. Refractive developments for individual DL-LIH monkeys



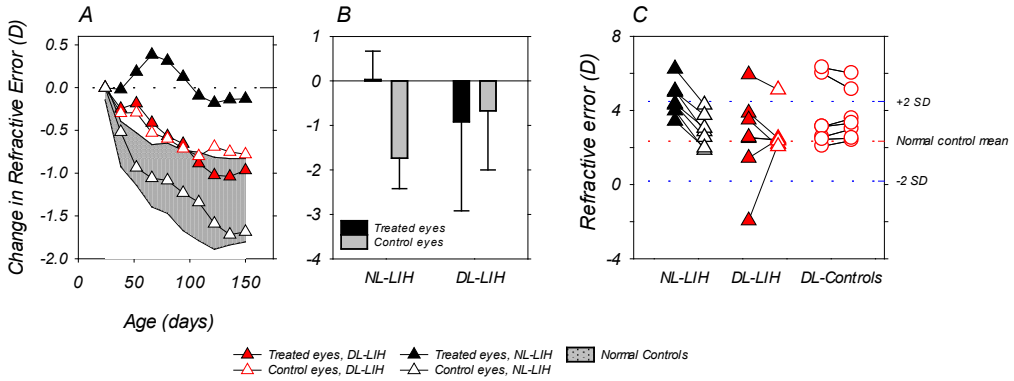
Refractive errors plotted as a function of age for the DL-LIH subjects. Red and white symbols represent data from the treated and control eyes, respectively; the grey thin lines in each plot represents the data from the right eyes of the normal control monkeys.

Figure 4-5. The development of compensating hyperopic anisometropias



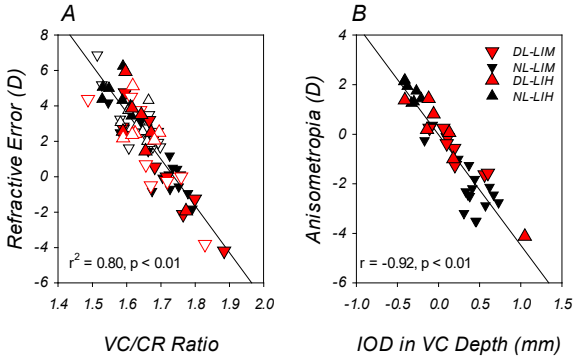
Anisometropia (treated eye ametropia – control eye ametropia) plotted as a function of age for the NL-LIH (panel A) and DL-LIH (panel B) groups. The circular symbols with error bars in each panel represent the mean ± SD in anisometropia obtained at the end of the lens-rearing period.

Figure 4-6. Refractive changes and end-of-treatment refractive errors for the LIH monkeys



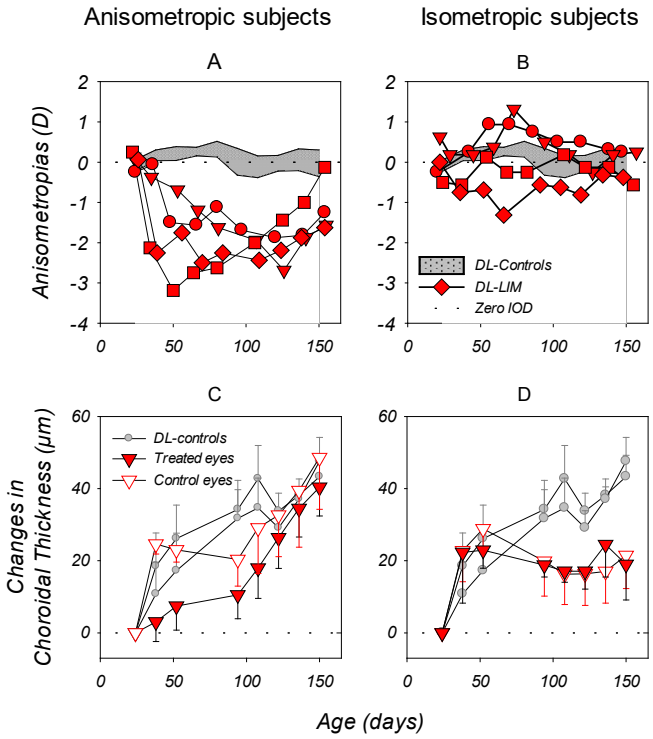
Panel A. Mean changes in refractive errors plotted as a function of age for the treated and control eyes of DL- and NL-LIH monkeys. To calculate the mean changes, data were linearly interpolated to an age series (range between 24-150 days, inclusive) that was equally spaced at 2-week intervals. The mean changes in the treated and control eyes of the DL-LIH monkeys showed no signs of compensation. Filled symbols: treated eye; open symbols: control eyes; red symbols: DL-subjects; black symbols: NL-subjects. Panel B. Mean ± SD changes in refractive errors obtained on the last day of the lens-rearing period. Error bars are not included in panel A for clarity. Panel C. Refractive error obtained at the end of the lens-rearing period for both eyes of the lens-treated monkeys and for control monkeys at ages corresponding to the end of lens-rearing period.

Figure 4-7. Relationship between refractive errors and axial dimensions



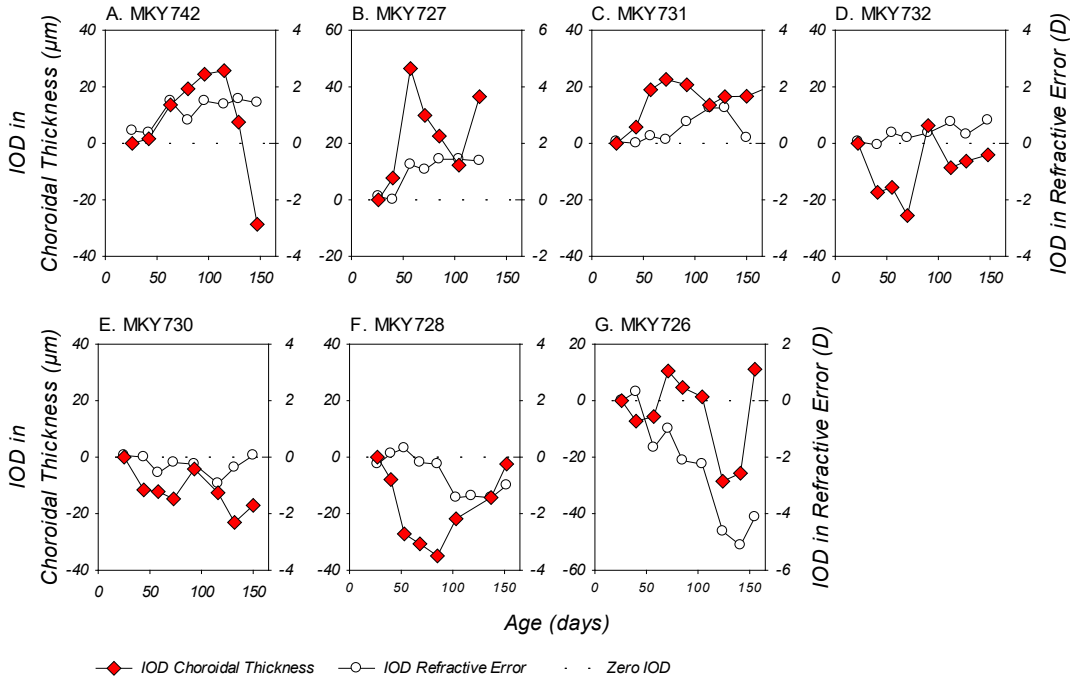
Panel A. Refractive errors obtained at the end of the experiment plotted as a function of the vitreous chamber to corneal radius ratios (VC/CR ratio). The filled and open symbols represent the treated and control eyes of the DL- (red) and NL-subjects (black), respectively. Panel B. Anisometropia obtained at the end of the experiment plotted as a function of the interocular difference in vitreous chamber depth for individual NL and DL animals (treated eye – control eye).

Figure 4-8. The relationship between the development of LIM and relative choroidal thickness changes



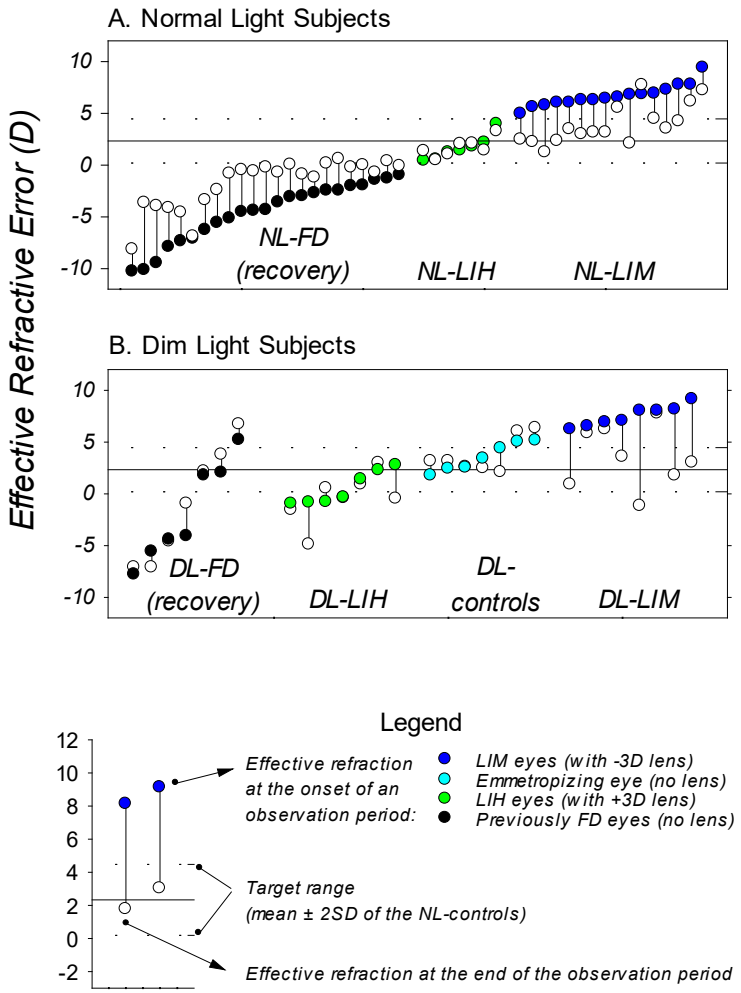
The degree of anisometropias (treated eye ametropia – control eye ametropia; panels A and B) and the mean (± SEM) changes in choroidal thickness (specified relative to the onset of the experiment) in the treated (filled symbol) and control eyes (open symbol) plotted as a function of age for DL-LIM monkeys that had developed compensatory anisometropia at any stage of the experiment (monkeys 706, 679, 687 and 688, left column) and those that remained isometropic throughout the experiment (monkeys 682, 683, 677 and 678, right column). The shaded areas in panels A and B represent the 95% confidence range of IODs in refractive errors for DL Controls. The grey lines and symbols in Panels C and D represent the choroidal thickness in the two eyes of the DL controls.

Figure 4-9. The relationship between the development of LIH and relative choroidal thickness changes



Changes in the interocular differences (treated eye – control eye) in sub-foveal choroidal thickness (red symbols) and refractive error (white symbols) plotted as a function of age for individual DL-LIH monkeys. The dashed line in each panel represents zero IODs in the changes of refractive error and choroidal thickness. Choroidal thickness development, at least in the early- and mid-stages of the lens-rearing period, was appropriate for the direction of refractive changes, i.e. monkeys that did not successfully compensate for imposed myopic defocus exhibited relative choroidal thinning instead of thickening.

Figure 4-10. Effects of dim light on visually regulated refractive development



Effective refractive error obtained at the onset (color-coded symbols) and end (open symbols) of the observation period for individual monkeys. **Panel A**: In normal light, the effective refractive states of monkeys systematically changed towards a target range. **Panel B**: DL monkeys did not show systematic changes in refractive error analogous to that illustrated in panel A. Data are presented for eyes that were permitted visual experience that would support visual regulation under normal light, i.e., the treated eyes of lens-wearing monkeys, the right eyes of the control monkeys (She *et al.*, 2020), and the diffuser-treated eyes of the FD monkeys at the time of removal of diffusers (i.e., the onset of recovery period. She *et al.*, 2021). Color-coded symbols: treated eye of LIH (green) and LIM (blue) monkeys at the onset of lens

treatment (approximately 24 days) and the right eyes of monkeys reared with unrestricted vision at ages that corresponded to the onset of lens-treatment (cyan); black symbols represent data of FD monkeys obtained at the onset of the "recovery" periods (approximately 150 days). Open symbols: data obtained from lens-treated monkeys at the end of the lens-rearing period (approximately 150 days for LIH monkeys and 135 days for and LIM monkeys), at ages that correspond to the end of lens-rearing period (for DL-controls), or at the end of the recovery period (300 and 350 days for NL- and DL-FD monkeys, respectively). These observation periods were within the age range in which refractive changes associated with visually regulated refractive development could be observed under normal ambient lightings. The vertical lines connect the data from the same eye to help identify the direction of refractive change.

Chapter 5.

General conclusion

The main findings of the three experiments are summarized below:

Experiment 1 (Chapter 2) showed that dim ambient lighting did not cause any instances of myopia in emmetropizing infant monkeys, but increased the inter-individual variability and the interocular differences in refractive error. In addition, the probability that monkeys would exhibit the normal age-related reductions in hyperopia was reduced. These observations suggested that dim light does not have a systematic myopiagenic effect for non-human primates that are undergoing emmetropization. The reduced probability of successful emmetropization suggested that the dim light might reduce the efficacy of regulatory mechanisms that are important for emmetropization.

Experiment 2 (Chapter 3) showed that dim light rearing did not affect the development of axial FDM, indicating that dim ambient lighting does not affect the unregulated, "intrinsic" ocular growth. In addition, dim light interfered with the visually driven recovery from this condition; the failure to recover was associated with an absence of the typical defocus-induced relative choroidal thickness changes, suggesting that the probability of visually regulated recovery is reduced under dim light. These findings indicate that dim ambient lighting is not a stimulus for myopia, per se. It does alter refractive development through its influence on the visual mechanisms that normally regulate refractive development.

Experiment 3 (Chapter 4) showed that dim light disrupted the typically consistent compensation to lens-imposed monocular defocus. This effect was qualitatively consistent with the results observed in the investigations of emmetropization and the recovery from FDM under

dim ambient lighting, and was also associated with an absence of defocus-induced relative choroidal thickness changes, indicating that the efficacy of the feedback-control mechanisms that operate to optimize the refractive state was reduced. In particular, a reduced responsiveness to myopic defocus led to the increased occurrence of myopia.

The results of these three experiments demonstrated that dim light mainly influences the efficacy of regulatory mechanisms that operate on visual signals to control ocular growth, thereby reducing the probability of normal refractive development. Although this effect could cause myopia in some cases, dim light per se did not produce any systematic myopiagenic effects.

Light-level response patterns and an updated model of light-level effects on axial refractive development

5.1.1 Introduction

Norton and Siegwart Jr. (2013) reviewed the findings on the effects of ambient lighting levels on refractive development. In addition to summarizing the myopia protection associated with elevated lighting levels, the review also laid out a model that aimed to explain how ambient lighting levels affect refractive error and ocular growth. The speculative model poses that retinal dopaminergic activity acts as a biological representation of ambient illumination, through which high ambient lighting slows normal refractive development, reduces the efficacy of myopiagenic stimuli, and vice versa. From a physiological perspective, this proposition is significant in that it suggests the myopia protection associated with elevated lighting is not a dichotomic effect; instead, it is part of a collection of graded changes in the course of refractive development in response to different ambient lighting levels. It is also significant with respect to understanding how myopia develops in humans, because a graded response characteristic predicts that lower ambient lighting levels should cause relative myopic changes.

However, not all studies support this hypothesis. For example, studies in our lab showed that emmetropization and negative lens-compensation in monkeys did not respond to elevated (Smith III *et al.*, 2012; Smith III, Hung, Arumugam, & Huang, 2013) or reduced ambient lightings (She *et al.*, 2020; also see Chapter 4) in a systematic manner as did chicks. In addition, monkeys showed higher variability and deviations from normal optically driven development patterns under reduced ambient lighting levels (She *et al.*, 2020, 2021; also see Chapter 4). The initial graded response proposition is not sufficient to explain these observations, nor is it sufficient to reconcile these observations with the previous ones in chicks. The purpose of this

chapter is to illustrate and compare the light-level-response characteristics of refractive development in the two well-studied species (rhesus monkeys and chicks), to put forward some speculations concerning the possible basic actions of ambient lighting levels, and to discuss the factors that might contribute to the observed light-level effects.

5.1.2 Comparison of light-level responses between chicks and monkeys

Figure 5-1 compares the developmental time course of normal emmetropization, negative-lens compensation, and FDM in chicks and rhesus monkeys. For lens-treated and diffuser-treated animals, the treated-eye refractions, instead of interocular differences in refraction, are illustrated. For each of the experimental models of ametropia, monkey data are available for three different ambient lighting levels ("elevated" levels at ~26,000 lux, typical laboratory levels at ~500 lux, and "reduced" levels at ~50 lux) (She *et al.*, 2020, 2021; Smith III *et al.*, 2012; Smith III, Hung, Arumugam, & Huang, 2013). For the chicks, data were replotted from the studies of Ashby and Schaeffel (2010), Cohen *et al.* (2011), and Karouta and Ashby (2015).. For form-deprivation myopia (FDM), we included the study of Ashby *et al.* (2009) on the development of FDM under a reduced ambient lighting level (50 lux), which employed a cross-sectional design and a shorter experimental period than the parametric longitudinal investigation of elevated lighting levels by the same group (Ashby *et al.*, 2015) (see Figure 5-1F).

Emmetropization. The rate of age-related reductions in hyperopia and the target refractive error in chicks (Figure 5-1A) were varied systematically with ambient lighting intensity. Elevated ambient lighting (15,000 lux) that was maintained continuously during the daily light-on phase was associated with slower reductions in hyperopia throughout the course of experiment, whereas reduced ambient lighting (50 lux) produced the opposite effect. The rate alterations appeared to increase over time and eventually caused an obvious light-level-

associated refractive difference in the animals reared under these two lighting levels. In monkeys (Figure 5-1B), a 6-hour daily exposure to elevated ambient lighting levels (26,000 lux) did not slow or accelerate the reductions in hyperopia. However, reduced ambient lighting levels (50 lux) increased the variability in refractive development; the pattern of average refractive error change did not conform to the typical pattern of emmetropization.

Negative lens-compensation. In chicks (Figure 5-1C) the rate of compensation for negative-lens-imposed defocus was inversely associated with the ambient light intensity. This association was observed even within a very short experimental period (5 days). Similar to emmetropizing eyes, the negative-lens-treated eyes showed slower reductions in hyperopia under elevated ambient lighting levels; however, the rate of reductions in hyperopia increased after the initial slowing and these eyes eventually fully compensated for the imposed defocus. Similar effects were also reported for tree shrews (Norton and Siegwart Jr., 2013). In monkeys (Figure 5-1D), 6-hour daily exposures to elevated ambient lighting levels (26,000 lux) did not slow the treated eye's refractive development. In addition, reduced ambient lighting also did not exacerbate the reductions in hyperopia in the early stages of lens-rearing; rate differences from those in typical lighting level only developed during the late stage of lens rearing. Hammond and Wildsoet in chicks (2012) did not find significant difference in the course of compensating changes negative lenses between subjects reared under 20 and 200 "chick lux" over a 7-day period. However, they found that chicks developed more relative myopia and higher variability in refractive error in response to monocular +20D lens under 20 "chick lux" ambient lighting, in comparison to that under 200 "chick lux" ambient lighting.

Form-deprivation myopia. In chicks (Figure 5-1E) the phenomenon of FDM was altered by ambient lighting levels in a manner that was very similar to those observed during emmetropization and negative-lens compensation. For the form-deprived eye, higher ambient

lighting levels were associated with slower myopia development. The changes in treated eye refractive error had a strong linear correlation with ambient lighting levels on a logarithmic scale. However, in a separate study (Ashby *et al.*, 2009), reduced ambient lighting levels (50 lux) did not change the final refractive error in the treated eyes of form-deprived chicks (the triangles in Figure 5-1E. Note that the data symbols are largely overlapped). In monkeys (Figure 5-1F), elevated ambient lighting level reduced the average amount of FDM and produced sustained hyperopic shifts in many treated eyes, but reduced ambient lighting did not change the developmental course of FDM.

5.1.3 Axial nature of refractive error: the commonality in light-level effects

Although the light-level-response patterns were not always consistent between chicks and monkeys, in both species the observed refractive-error alterations were, at least partially, associated with differential vitreous chamber elongation. For example, under elevated ambient lighting levels, the relative hyperopic endpoints for emmetropization (Cohen *et al.*, 2011 and 2012) and slower negative-lens compensation in chicks (Ashby *et al.*, 2010) were associated with slower axial growth. In monkeys, all of the observed refractive changes associated with reduced or elevated ambient lighting were associated with direction-appropriate changes in vitreous chamber elongation (She *et al.*, 2020, 2021; also see Chapter 4). The consistent association between difference in axial elongation and refractive error indicates that the common final product of ambient-lighting-level effects, should they occur, was alteration in the axial ocular growth rate, i.e. there was a common axial nature for most of the observed light-level effects.

Ocular growth associated with normal refractive development is achieved through a series of biochemical and biomechanical changes in the sclera, a process that is regulated by the functional signal produced by local retinal mechanisms that utilize visual environmental cues

to optimize optical state (Troilo *et al.*, 2019). The common axial nature of refractive effects associated with ambient lighting levels implies that simple mechanistic actions, potentially in as few as one part of the regulatory signaling cascade, could elicit the observed alterations; in this respect, the existing evidence supports the speculated graded response model, which states that light-induced changes in retinal dopamine levels mediate the graded refractive responses (but see 5.1.8.1).

However, axial elongation may not always be the sole contributor to the light-level-associated refractive effects. For example, the myopias associated with low ambient lighting levels in emmetropizing chicks were associated with thicker crystalline lenses and greater corneal powers (Cohen *et al.*, 2011). At present, there is a paucity of data on the effects of ambient lighting levels on the eye's optical components in chicks. Nevertheless, this observation suggests that the development of the corneas and lenses in certain species might be susceptible to lighting level differences, which could result in alterations in the effective refractive state at the end of or during refractive development.

For humans, vitreous chamber elongation is the predominant determinant of the eye's refractive state (Troilo *et al.*, 2019). To extrapolate the results of animal studies to humans, careful analyses of the role of axial development in refractive error is essential. In this respect, some modified ocular dimension metrics (e.g. vitreous chamber to corneal radius-ratio, or VC/CR ratio) might be of value in analyzing the ocular dimension changes that contribute to light-level effects.

5.1.4 Relevant levels of ambient illumination

The occurrence and interpretation of light-level effects are largely influenced by the ambient lighting levels of interest and the reference or baseline lighting levels. What is the range of

ambient lighting levels that is relevant with respect to the translational application of animal studies to humans? In the studies of chicks, a clear pattern of light-level-dependency was observed over a range of ambient lighting levels between 50 and 40,000 lux (Cohen *et al.*, 2011; Karouta & Ashby, 2015). The term "elevated ambient lightings levels" has typically referred to lighting levels that were above 10,000 lux. For humans, ambient lightings that are at or above the "elevated" levels in experimental settings are commonly encountered in outdoor environments, but are normally not encountered indoors with typical artificial illumination[1]. On the other hand, concerns with respective to the clinical relevance of animal studies also might be directed at the ambient lighting levels that were supposedly representative of "normal" and "reduced/low" ambient illuminations. In this regard, recent studies have shown that the ambient lighting levels employed in animal refractive error studies are common for humans (Ostrin, 2017; Wen *et al.*, 2019, 2020). For example, Wen *et al.*, using a device that measures light levels in a way that is presumably more closely related to visual experience, showed that the average ambient lighting levels in an urban setting fluctuate around 500 lux depending on the nature of visual activity (schoolwork, afterschool activity, or weekend) (Wen *et al.*, 2019). Another study showed that ambient lighting levels associated with visual environments could vary from ~300 lux in the afternoon to under 10 lux at night; in addition, the illuminance levels in visual environments that are presumably associated with heavy near work could be lower in myopic children than those in non-myopic children by approximately 200 lux (Wen *et al.*, 2020). These findings indicate that the ambient lighting levels associated with the visual experience in children are often similar to the "normal" levels and sometimes close to the "reduced" levels employed in animal studies. In this respect, the "normal" lighting levels

[1] Based on the following guidelines/standards for interior lighting: ANSI/IESNA RP-1-12; AS/NZS 1680.2.3:2008; ISO 8995-1:2002(E)/CIE S 008/E:2001

commonly employed in animal experiments appear to be an appropriate reference for the refractive effects of incremental/decremental changes in ambient lighting levels.

Currently, there is no definitive evidence on the operating range of ambient lighting levels for the mechanisms responsible for the observed graded refractive response. Karouta and Ashby (2015) speculated that, at 40,000 lux, the protection of high ambient lighting against FDM could be near-complete. Note that this highly effective "elevated" laboratory ambient lighting level remains lower than the ambient lighting levels typically associated with outdoor environments, which implies that there is a possible ceiling for any graded ambient lighting level effects associated with outdoor environments. In addition, for monkeys, daily periods of exposure to elevated ambient lightings at 26,000 lux dramatically reduced the likelihood that an infant monkey would develop FDM and resulted in moderate hyperopic shifts in the form-deprived eyes of the majority of monkeys (Smith *et al.*, 2012), suggesting that the ambient lighting levels at which the ceiling occurs might be lower in monkeys than in chicks. On the other hand, although some studies showed that reduced ambient lighting (~50 lux) failed to systematically change the degree of FDM in comparison to "normal" lighting levels (Ashby *et al.*, 2009; She *et al.*, 2021), whether this ineffectiveness represented the lower end of the response range is debatable. Arguments in this respect were supported by some early findings that very low ambient lighting levels could produce the predicted myopic changes (Bercovitz *et al.*, 1972; Lauber & Kinner, 1979). It should be noted, however, that these very low ambient lighting levels have strong physiological effects beyond refractive regulation. For chicks, low intensity ambient lighting (<20 lux) is a potent exogenous modulator that induces changes in weight gain, breeding, and behavior (Blatchford *et al.*, 2009; Deep *et al.*, 2010; Newberry *et al.*, 1988). In humans, low daytime ambient lighting levels (25 lux) affected the successful entrainment of biological circadian cycle (Duffy & Czeisler, 2009). The possible involvement of systemic effects and/or mechanisms, particularly those that involves circadian rhythms,

suggests that the refractive changes caused by very low ambient lighting conditions could involve systemic or non-optical mechanisms. At present, there is no evidence that the range of graded light-level responses for FDM extends to an ambient lighting level of 50 lux, which might be due in part to its small decremental change from the "normal" reference level. For visually regulated refractive development, the studies of Cohen *et al.* (2011 and 2012) were the only long-term longitudinal investigations that indicated graded responses could occur over a similar range of ambient lightings; however careful analyses should be performed to determine the factors that contributed to the observed refractive changes.

It should be recognized that very few of the light-level studies measured light-induced changes in pupil size, which affects the retinal illumination that eventually activates any ocular mechanism responsible for the observed light-level effects. Therefore, the actual operating range of light-level responsiveness remains unclear. Finally, it is also important to know whether humans and laboratory animals have similar responsivity within the relevant range of ambient lighting levels. Unfortunately, how human refractive development responds to ambient lighting levels have not been rigorously investigated.

5.1.5 Ambient lighting levels modulate intrinsic ocular growth rates and play a permitting role in visual regulation

The centerpiece of the graded-response model is that the course of refractive development is associated with ambient lighting levels. Accordingly, given an appropriate range of lighting levels and sufficient incremental/decremental differences, ambient-lighting-level changes could alter refractive development in a consistent manner. This prediction was substantiated by the strong, inverse light-level dependency of FDM observed in chicks (Karouta & Ashby, 2015). These light-dependent alterations were discernable in the early stage of form deprivation and were systematic in that they were consistently directional with respect to lighting level

differences. A similar dependency might also exist in monkeys (Smith III *et al.*, 2013;), tree shrews (Siegwart *et al.*, 2012), mice (Chen *et al.*, 2017), and guinea pigs (Zhang and Qu, 2019), species in which FDM was also reduced by high ambient lightings. In association with the preceding discussion, form-deprivation could be considered a special and possibly the simplest case with respect to ocular elongation regulation (an "open-loop" condition) (Schaeffel & Howland, 1991). Due to the elimination of meaningful visual input, the systematic response of the phenomenon of FDM indicates that lighting levels systematically modulated the rate of ocular elongation driven by the natural tendency of the eye to grow, i.e., the intrinsic axial growth rate of the eye.

Ambient lighting levels have also been shown to systematically alter the course emmetropization in chicks (Cohen *et al.*, 2011, 2012) in a manner that is similar to that in FDM (Karouta & Ashby, 2015). However, due to the presence of active visual regulation, it is not clear which part(s) of the signaling cascade was affected. One possibility is that ambient lighting levels modulated the gain of optical signals that drive emmetropization, with elevated lighting levels reducing the gain for hyperopic defocus, resulting in a decrease in the magnitude of the functional output that controls ocular elongation, and consequently slowing the expected age-related reductions in hyperopia in neonates, resulting in relative hyperopic shifts. In this respect, the fact that elevated ambient lighting levels produced relative hyperopias in both negative- and positive-lens-rearing chicks indicates that light-levels could modulate the gain of optical signals in a sign-dependent manner: elevated ambient lighting levels increase the gain for myopic defocus, but reduce the gain for hyperopic defocus, and *vice versa*. A simpler alternative is that the light-level modulates the intrinsic ocular growth rate, which is independent of and thus might co-exist with active defocus-driven visual regulation. Specifically, a slower intrinsic growth under elevated lighting levels makes the functional "grow" signals associated with hyperopic defocus less efficient, but makes functional "stop"

signals associated with myopic defocus more efficient, hence the sign-dependent alterations. We speculate that, due to the benefit of simplicity, the interaction between intrinsic growth-rate modulation and active defocus-driven visual regulation might be the primary mechanism for the light-level-associated systematic changes in emmetropization and lens compensations observed in chicks.

We found that the same reduced ambient lighting levels that induced myopias in emmetropizing chicks caused variable, but significant deviations from the typical course of defocus-driven refractive development in infant monkeys (see Figure 4-1, Figure 4-4, and Figure 4-10). In comparison to the systematic changes reported in chicks, these alterations lack uniformity in direction and were often in the opposite direction to the changes observed in chicks. This "disruptive" pattern cannot be adequately explained as simple graded responses to lighting levels. We speculated that it might represent an effect that differs fundamentally from the systematic effects described above (see Chapter 2 – Chapter 4). It is possible that reduced ambient lighting compromised the operational efficacy of the visual mechanisms that normally optimize refractive state, thereby reducing the probability of successful, optically driven refractive development. In support of this view, for both chicks and monkeys, longitudinal evaluations of choroidal thickness have revealed an association between the absence of normal vision-induced relative choroidal thickness changes and the failures in developing compensatory anisometropias (Hammond and Wildsoet, 2012; Chapter 4). Since monkeys emmetropized and compensated to lenses normally at "typical" and "elevated" ambient lighting levels, the disruptive effects associated with "reduced" ambient lighting suggest that an ambient lighting level that is at or above typical laboratory level is a prerequisite for successful optically driven refractive development. If correct, this speculation implicates a permitting role of ambient lighting levels in visual regulation.

In summary, the systematic effects of ambient lighting levels and the disruptive effects of reduced ambient lighting represent different actions of light levels. On the one hand, ambient lighting levels modulate the intrinsic rate of ocular elongation, producing systematic changes in the time course of axial refractive development. On the other hand, light level plays a permitting role in refractive regulation, with successful refractive regulation activity requiring an ambient lighting level that is at or above typical laboratory levels.

5.1.6 Visual regulation for ocular growth could mask the light-level modulation of intrinsic ocular growth rate

A difficulty in understanding the refractive responses to lighting levels is that the patterns of response to alterations in the ambient lighting levels were not always consistent between experimental manipulations. This was particularly significant for monkeys, where, for example, elevated lighting prevented FDM but not lens-induced myopia. In contrast, in chicks, the light-level-response patterns for normal emmetropization and lens compensation were somewhat similar in nature to those of FDM, i.e., elevated lighting promoted hyperopia and reduced the rate or degree of myopia (see 5.1.1.3).

Lens compensation could be considered a stress test for emmetropization (Norton and Siegwart Jr., 2013). In this respect, for monkeys, the presence or absence of meaningful visual feedback that normally guides emmetropization appeared to be the key distinction between different light-level response patterns. When visual experience supports regulatory activity, such as those associated with unrestricted vision or monocular lens-wear, ambient lighting levels (i.e., elevated ambient lighting) did not produce significant systematic effects that were similar in nature to those observed in FDM. In association with the preceding reasoning, it appears that the difference in light-level-response patterns between monkeys and chicks reflects the extent to which active visual control of ocular elongation could mask the refractive

effects of light-level modulations in intrinsic ocular growth rate. Note that the apparently complete "masking" for rhesus monkeys undergoing emmetropization and negative-lens compensation (Smith *et al.*, 2013) were probably not due to insufficient increases in lighting levels or lower responsivity to brighter ambient lighting, because the same elevated ambient lighting levels produced substantial and persistent reductions in FDM (Smith *et al.*, 2012). In this respect, it is possible that the weighting of light-level modulation in controlling ocular elongation is lower in monkeys than in chicks. If correct, this weighting difference could be the underlying cause of the difference in light-level effects between non-human primates and avian species.

5.1.7 An updated view of light-level effects

We speculate that ambient light levels influence two aspects of axial refractive development. On the one hand, a conserved ocular mechanism, of which the response characteristics are presumably similar between species, responds to ambient lighting levels to modulate the intrinsic ocular growth rate. As a consequence, light levels produce a graded effect on the intrinsic ocular growth rate, as reflected by the observations of FDM. This graded effect is independent of and could interact with defocus-driven visual regulation, but substantial inter-species differences apparently exist in the weighting of the light-level modulation in affecting the final or overall ocular growth. For avian species, light-level modulation has a higher weighting on the final eye growth control, such that the efficiencies of the functional signals produced by optical "stop" and "go" signals could be altered (result in rate-modulation) or overridden (result in end-point modulation. Cohen *et al.*, 2011 and 2012) by light-driven changes in the intrinsic growth rate, so that optically driven refractive development would exhibit similar light-level-response characteristics as FDM (Ashby *et al.*, 2015; Cohen *et al.*, 2011 and 2012; see Figure 5-1). For non-human primates, light-level-modulation has a lower

weighting in controlling ocular growth. When visual experience permits defocus-driven regulatory activity, the output of the visual regulation mechanisms, regardless of quality, dominates the course of axial refractive development (Smith *et al.*, 2013; She *et al.*, 2020; also see Chapter 4); light-level-modulations of intrinsic ocular growth rates only manifest when defocus-driven regulatory visual cues are absent or weak (Smith *et al.*, 2012; She *et al.*, 2021). On the other hand, it appears that ambient lighting levels determine whether the visual mechanisms can function at a high level of efficacy, at which eyes have a high probability to emmetropize successfully. Once ambient lighting intensity reaches the required level and optimal efficacy in refractive regulation is permitted, further elevation in lighting level do not produce more refractive effects through this non-graded mechanism. Because emmetropization and lens-compensation under normal or elevated ambient lighting levels showed signs of normal defocus-driven optical regulation, the ambient lighting intensity required for optimal functionality, or a "critical" ambient lighting level for this non-graded, permitting role of ambient lighting levels, is presumably between common laboratory lighting level and the reduced ambient lighting levels employed in the studies in monkeys (~50 lux).

5.1.8 Other factors associated with ambient lighting effects

5.1.8.1 Pupil diameter

Pupil diameter has a significant impact on retinal illumination. For example, in comparison to normal ambient lighting (~300 – 500 lux, pupil size = 3.8 – 4.0 mm) (Smith *et al.*, 2012; She *et al.*, 2020), the pupil diameters in monkeys decreased by 58% (to 1.6 mm) under elevated ambient lighting (Smith *et al.*, 2012) and increase by 30% - 44% (to ~5 mm) under ~50 lux ambient lighting (She *et al.*, 2020 and 2021). Assuming that the pupil is circular, these diameter differences would result in pupil area differences of -82% under elevated ambient lighting and +107% under reduced ambient lighting. At this magnitude, pupil area is a significant factor in

estimating the "true" relationship between light intensity and refraction, which could provide a theoretical basis for determining the minimum ambient lighting levels that might be clinically pro-hyperopia/anti-myopia. Unfortunately, very few of the existing lighting level studies measured pupil diameter changes, and none have quantified the relationship between pupil size, retinal illuminance, and refractive development.

Pupil size changes also alter the depth-of-focus (DOF) and thus the efficiency of optical control of refractive development. It is speculated that the increase in DOF that accompanies the pupil constrictions under high ambient lighting might reduce the magnitude of hyperopic blur (Ashby *et al.*, 2010; Smith III *et al.*, 2013) to a point that the effective refractive state becomes insufficient to further stimulate ocular elongation (Smith III *et al.*, 2013). However, light-level-differences must be substantial to produce any optical effect that is sufficiently sizable to be relevant in refractive regulation. In humans, decreases in pupil diameter (and thus increases in DOF) were found to be non-linear on a logarithmic scale over a range between 2 – 20,000 trolands and pupil constriction only became significant after retinal illuminance surpasses 200 trolands (Barrionuevo & Cao, 2016). In chicks, the non-linearity in pupil-light reflex was very similar to that in humans (Schaeffel *et al.*, 1986). Therefore, pupil size might have an optical effect in the high-light-associated relative hyperopias in animals and in the protections against myopia associated with more outdoor activity in children, but its role in the myopia protections associated with mild elevations in indoor ambient lighting levels should be very limited (Hua *et al.*, 2015).

5.1.8.2 Biochemical mediators

To date, it is not clear what biochemical changes mediate the modulatory effects of ambient lighting intensity. The initial propositions that retinal dopaminergic activity mediates the observed graded response to ambient lighting levels (Norton & Siegwart Jr., 2013) was

challenged by some recent studies. For example, the protections against myopia associated with outdoor rearing were not correlated with retinal dopamine nor 3, 4-dihydroxyphenylacetic acid (DOPAC) levels (Stone *et al.*, 2016). In addition, mice reared under photopic, mesopic, and scotopic ambient lighting had similar retinal dopamine levels (Landis *et al.*, 2021). These results suggest that other factors, such as temporal adaptation of the dopaminergic system and inter-species/inter-individual difference in dopaminergic activity interfered with the simple quantitative relationship between lighting level, retinal dopamine level, and refractive development. It is also possible that biochemical gradients exist across the retina-choroid-sclera complex, and a quantitative correlation could be observed in the choroid and/or sclera.

We previously speculated that low light-level-induced adaptive changes in retinal function incidentally changed the functional state of retinal mechanisms that are important for refractive regulation. We further speculated that retinal dopaminergic activity might play a role in this process; specifically, individual differences in dopaminergic activity might determine whether a specific lighting level supports the optimal functionality of refractive regulation (see Chapter 4). This speculation seemingly contradicts the existing knowledge that reduced dopamine level was initially found to be associated with FDM (Stone *et al.*, 1989) and was argued to play a more important role in FDM than in lens-induced myopias (Dong *et al.*, 2011). However, dopamine is also extensively involved in retinal function, and more importantly in their light-associated adaptive responses. In particular, Jackson *et al.* (Jackson *et al.*, 2012) showed that retinal dopamine is an important molecule in regulating contrast sensitivity and spatial resolution during light-adaptation. The close associations between dopamine and retinal functions that are presumably important for refractive regulation appears to support this view. Further investigations are required to determine the relationship between dopamine-mediated light adaptation and the efficacy of the mechanisms that normally regulated refractive development.

5.1.8.3 Choroidal response to lighting level and non-vitreous (non-scleral) components of refractive changes

Observations in chicks and monkeys both suggested that changes in ambient lighting levels could induce choroidal thickness changes. For example, chicks exposed to 6-hour of 15,000 lux ambient lighting levels on a daily basis exhibited small degrees of relative choroidal thinning (Lan *et al.*, 2013). Interestingly, in the same experiment, after the ambient lighting levels were returned to normal for 2 hours and then the animals had housed in darkness for another 2 hours, short-term exposure to elevated ambient lighting then produced "rebound" increases in choroidal thickness. As described in Chapter 2, monkeys reared under ~50 lux ambient illumination showed progressive choroidal thickening over the dim-light-rearing period (She *et al.*, 2020).

Factors associated with these choroidal thickness changes are not always clear. For monkeys, the progressive choroidal thickening was unambiguously associated with the lower ambient lighting levels during the daily light-on hours. For chicks, the tendency to thin after exposure to higher ambient lighting was also clear; however, the cause of the more significant rebound thickening was confounded by the time of the day at which the choroidal thicknesses were measured in chicks (after the animals had entered the normal dark phase of the daily light-dark cycle). Based on the experimental design, we speculate that the thickening could have been associated with the transition from high to low ambient lighting levels. Some observations seem to support an association between lower ambient lighting levels and thicker choroids. For example, the substitution of 150 lux night-time ambient lighting with of 1,000 lux ambient lighting reportedly caused reductions in sub-foveal choroidal thickness in humans (Ahn *et al.*, 2017). It should be noted, however, that other factors, such as disruptions in the circadian rhythm of choroidal thickness (Nickla *et al.*, 2002), might also contribute to the observed light-

induced choroidal thickness changes. At present, it might be prudent to speculate that, at the very least, both ambient lighting intensity and its temporal pattern of change could influence choroidal thickness.

If ambient lighting levels can induce choroidal thickness changes, it is important to determine whether the magnitude of these changes is sufficient to directly influence the eye's measured refractive state. In monkeys, the relative thickening induced by dim light (She *et al.*, 2020) was similar in magnitude to those induced by defocus under typical indoor lighting levels (Hung *et al.*, 2000), which does not significantly impact the eye's refractive state. In chicks, the magnitude of the observed rebound thickenings was greater than the thinning induced by bright lighting (Lan *et al.*, 2013), but were much smaller than those induced by other common visual manipulations (Wallman *et al.*, 1995; Wildsoet & Wallman, 1995). However, due to the potential of choroidal expansion as indicated by the vision-induced choroidal thickness changes, one could not exclude the possibility that more drastic changes in choroidal thickness could be elicited if different lighting paradigm were employed. In addition, choroidal thickness related changes in refractive state might augment the effects of any light-level-associated changes in corneal and lens power; given sufficient magnitude this summation could potentially mask the true relationship between refraction and axial elongation, and might alter the apparent time course of refractive development.

5.1.9 Conclusions and implications

This chapter described two possible basic actions of ambient lighting levels on refractive development. Specifically, ambient lighting level has a graded modulation effect on the eye's intrinsic growth rate, which could interact with defocus-driven visual regulation, and a non-graded, permitting role on the optical mechanism that is responsible for visual regulation. In comparison to that speculated by Norton and Siegwart Jr. (2013), the preceding reasoning states

that a graded effect exists, but is limited to the modulation of the intrinsic ocular growth rate, and that the observed systematic responses to alterations in ambient lighting levels might be related to inter-species differences in the weighting of light-level modulation on the final control of ocular elongation.

Being diurnal animals, chicks and monkeys are expected to frequently encounter high ambient lighting level in their natural habitats. The different weightings of light-level-modulation in eye growth control might firstly be features that suit their vision needs. On the one hand, for chicks and probably other diurnal birds, allowing high light to produce mild relative hyperopias increases the chance that their refractive errors fall within the hyperopic range at the end of emmetropization, which allows them to stay in-focus as needed by means of accommodation. On the other hand, a lower weighting of light-level-modulation in non-human primates could mean that ambient lighting levels are less likely to influence optically driven refractive development. This characteristic increases the probability that emmetropization mechanisms will optimize the eye's refractive state regardless of the ambient lighting levels and reduces the risk of high-light-associated hyperopia (Cohen *et al.*, 2011). In comparison to chicks, preventing excessive hyperopia is, to some extent, an advantage to non-human primates and probably humans: due to their long refractive development period, the amount of extra hyperopia resulting from a high weighting of light-level modulation could become considerable in relation to their amplitude of accommodation.

From a myopia-control perspective, the lower weighting of light-level modulations in non-human primates is both an advantage and a disadvantage. Although it allows access to higher ambient illumination to potentially protect developing eyes from myopia onset and development, the less prominent role of light-level-modulation in addition to a possible ceiling effects of intensity-dependency suggest that the total period of exposure must be substantially

long to produce a protective effect. In this perspective, children who start to access higher ambient light at earlier ages are more likely to be protected from future myopia onset.

A graded response to lighting levels predicts that lower ambient lighting levels could impose a relative risk of myopia in comparison to higher ambient lighting levels. This extrapolation is potentially important because human visual environments can often have ambient lighting levels that are below the levels required to support normal refractive development (Ostrin, 2017). Based on the above reasonings, the graded component of light-level effects might not directly produce myopia at a photopic illumination levels that are below typical laboratory lighting levels; however, there is an increased risk of myopiagenesis that is associated with a reduced efficacy of optical regulation involving myopic defocus (Chapter 4).

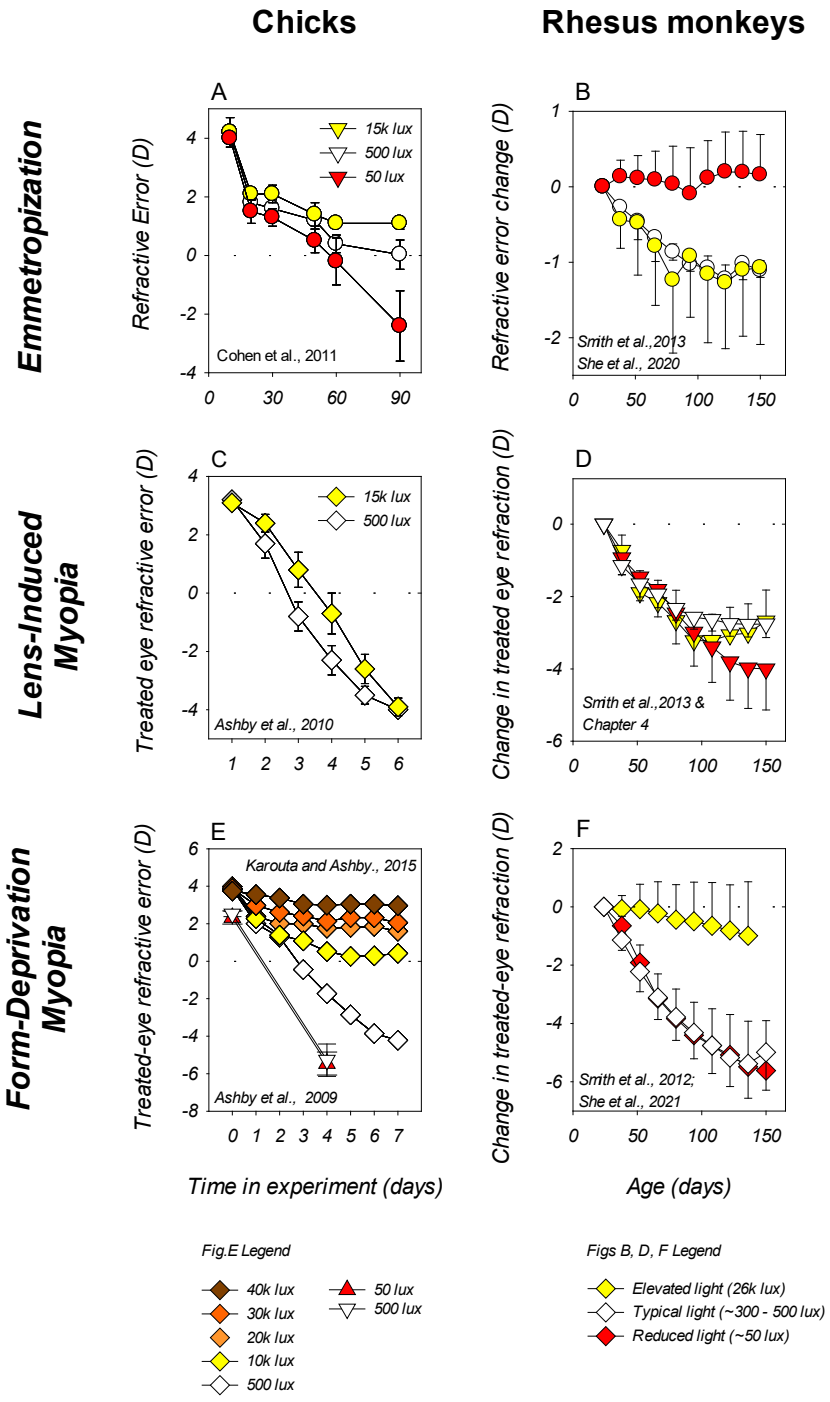
5.1.10 Limitations

Although the above propositions appear sufficient to reconcile some of the differences in the light-level-associated refractive effects, some response characteristics are still difficult to explain. For example, if, as speculated, high ambient lighting levels could only produce 1-2D of axial hyperopia in emmetropizing chicks (Norton & Siegwart Jr., 2013), how did it produce similar dioptric difference in the treated eyes of the negative-lens-reared chicks in only 3 days? (Ashby & Schaeffel, 2010) The fact that these responses were too rapid to be solely attributed to axial elongation suggests fast-responding mechanisms, such as choroidal responses to lighting levels, might be involved. It is also difficult to explain why elevated ambient lighting levels appeared to "modulate" the endpoint of emmetropization (Cohen *et al.*, 2011 and 2012), but did not change the endpoint of lens-compensations in a similar manner (which, as discussed, is essentially "with-the-lens" emmetropization) (Ashby *et al.*, 2010; Hammond and Wildsoet, 2012; Norton and Siegwart Jr., 2013). In these considerations, future studies should employ standardized protocols to avoid confounding factors associated with experimental design; in

addition, choroidal thickness and pupil size should be measured and their interactions with corneal power and axial dimension components should be analyzed to accurately interpret the results and correctly apply animal study findings to humans.

Tables and Figures

Figure 5-1. Refractive response profiles of chicks and rhesus monkeys



Refractive development of rhesus monkeys and chicks undergoing emmetropization, form-deprivation, or negative-lens-rearing under different ambient lighting levels. For the chicks (panels A, C, E), absolute refractive errors are plotted as a function of the number of days in the experiment. Data are replotted from Ashby *et al.*, 2009, Ashby *et al.*, 2010, Cohen *et al.*, 2011, and Karouta and Ashby, 2015. For monkeys (panels B, D, F), refractive error changes, specified relative to the baseline level, are plotted as a function of age. Data are replotted from Smith *et al.*, 2012; Smith *et al.*, 2013; She *et al.*, 2020 and 2021, and from Chapter 4.

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