Available online at www.sciencedirect.com
SCIENCE DIRECT®
Vision Research 46 (2006) 579–589
Vision Research
www.elsevier.com/locate/visres

Longitudinal changes of optical aberrations in normal and form-deprived myopic chick eyes

E. García de la Cera [a], G. Rodríguez [b], S. Marcos [a,*]

[a] Instituto de Óptica, Consejo Superior de Investigaciones Científicas, Madrid, Spain
[b] Instituto Universitario de Oftalmobiología Aplicada (IOBA), Universidad de Valladolid, Valladolid, Spain

Received 21 February 2005; received in revised form 13 June 2005

Abstract

We performed measurements of refraction (with retinoscopy), axial length (with ultrasound biometry) and ocular aberrations (with a custom-built Hartmann–Shack aberrometer) on seven awake White-Leghorn chicks occluded monolaterally with diffusers for two weeks. Treatment started on the first day after hatching (day 0) and measurements were conducted on several days between day 0 and 13. Non-occluded eyes experienced normal emmetropization (decreasing hyperopia at 0.2 ± 0.09 D/day and increasing axial length at 0.05 ± 0.03 mm/day), while occluded eyes developed axial myopia (1.50 ± 0.2 D/day and 0.12 ± 0.02 mm/day). Interocular differences in refraction and axial length by day 13 were on average 17.43 D and 0.86 mm, respectively. Monochromatic high order aberrations decreased with age in both eyes. Average RMS (for 1.5 mm pupil diameter) decreased from 0.11 ± 0.03 at day 0 to 0.06 ± 0.03 μm (day 13) in occluded eyes, and from 0.12 ± 0.05 to 0.03 ± 0.01 μm in non-occluded eyes. MTF-based optical quality metrics also show an improvement with age. However, while this improvement occurs in both eyes, after day 8 myopic eyes tend to show significantly higher amounts of aberrations (and consequently worse best-corrected optical quality) than normal eyes. The degradation imposed by aberrations is small compared to that imposed by defocus and the diffuser. These results suggest a decrease of aberrations during development which does not seem to be visually guided. Myopic eyes showed slightly worse optical quality than normal eyes, suggesting that the geometrical changes resulting from excessive ocular axial growth also affect the optical quality of the ocular components.

Keywords: Emmetropization; Experimental myopia; Chick model; Ocular aberrations; Form-deprivation; Optical quality

1. Introduction

There is compelling evidence, mostly from animal models, that the absence of a normal visual experience in the early stages of development compromises emmetropization, i.e., the normal ocular growth aiming at matching axial length of the eye to its optical power and achieving focused images on the retina. For a review see (Smith, 1998; Wallman, 1993; Wildsoet, 1997). It is well established that visual form deprivation, as well as other ways of altering the visual environment, produces axial elongation and myopia in a variety of species. The chick has been an extensively used animal model, and many studies have shown that degraded retinal image quality causes ocular elongation and myopia, particularly when the treatment is performed in neonates or young animals. Myopia development has been achieved with lid closure (Yinon, 1984), deprivation of form vision by placing opaque or translucent goggles in front of the eye (Hayes, Fitzke, Hodos, & Holden, 1986; Troilo & Wallman, 1991; Wallman & Adams, 1987), or restricting the contrast and spatial frequencies of the visual environment (Schmid & Wildsoet, 1997).

* Corresponding author.
E-mail address: susana@io.cfmac.csic.es (S. Marcos).

0042-6989/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.visres.2005.06.012

With the previous methods the eye growth control system runs open-loop with no possible feedback. Myopia has also been achieved by placing negative lenses in front of the animal's eye. In this case, the eye adjust its growth to compensate for the imposed defocus (Kee, Marzani, & Wallman, 2001; Schaeffel, Glasser, & Howland, 1988). It has also been observed that when normal vision is restored, even for short periods of time, the myopia tends to regress (Troilo & Wallman, 1991). While many studies have been performed on chicks, the impact of visual experience on normal eye growth has also been demonstrated in primates (Troilo, Nickla, & Wildsoet, 2000; Wiesel & Raviola, 1977). Also, pathology-related form deprivation in human infants (by eyelid closure, congenital cataracts or corneal opacities) has been associated to the development of myopia.

While conventional refractive errors are the most common source of optical degradation, human eyes (and possibly other species') also suffer from optical imperfections (called high order aberrations) that degrade the retinal image. The investigation of possible relationships between optical aberrations and myopia seems suggestive, in particular since the causes of myopia are not well understood. In recent years, aberrometers have been developed allowing rapid assessment of optical image quality beyond conventional refractive errors (He, Marcos, Webb, & Burns, 1998; Liang, Grimm, Goelz, & Bille, 1994; Moreno-Barriuso, Marcos, Navarro, & Burns, 2001). Several works have shown that myopic eyes show higher amounts of higher order aberrations than emmetropic eyes (Atchison, Collins, Wildsoet, Christensen, & Waterworth, 1995; Paquin, Hamam, & Simonet, 2002). These studies show a co-variability, but not a cause-effect relationship. Some results suggest that the constant degradation of the image quality produced by increased aberrations could disrupt the emmetropization process. For example, a recent study (Buehren, Collins, & Carney, 2003) found that sustained reading (usually attributed as a cause of myopia) produced a significant increase in corneal aberrations, which lasted well after completing the near work task. Also, results from clinical trials have shown that rigid contact lenses reduced the progression of myopia in children and adolescent subjects, compared to controls wearing soft contact lenses or spectacles (Khoo, Chong, & Rajan, 1999; Perrigin, Perrigin, Quintero, & Grosvenor, 1990). Interestingly, aberration measurements on rigid gas permeable (RGP) contact lens wearers with and without the contact lens on have shown the capability of RGP contact lenses to correct for significant amounts of high order aberrations (Dorronsoro, Barbero, Llorente, & Marcos, 2003). While those results are suggestive, there is no definite proof that aberrations could be a cause of myopia nor that cancelling aberrations could be a potential way of reducing excessive ocular growth. On the other hand, it has been argued that the presence of aberrations may provide clues to determine the sign of defocus, since interactions between high order aberrations and defocus (and as a consequence retinal image quality) change with the sign of defocus, and that these effects may be important in the emmetropization process (Wilson, Decker, & Roorda, 2002). Alternatively, the ocular enlargement of myopic eyes (and therefore different geometrical properties of the ocular components) could be the reason for the increased amount of aberrations found in myopic eyes. The question is whether the increased optical aberrations in myopic eyes are a cause or a consequence of myopia.

Unlike studies in animal models, to test cause-effect relationships in humans is complicated, due to the time cost of longitudinal studies and impossibility of intervening the ocular optical properties in infants. While chicks have been widely used as animal models of myopia, their optical quality has not been studied experimentally in much detail. In most studies, modelling and conclusions assume diffraction-limited optics. Coletta, Marcos, Wildsoet, and Troilo (2003) reported optical quality (in terms of modulation transfer function) of normal and myopic chick eyes using a double-pass method. To our knowledge, two studies presented in scientific meetings (Campbell, Hunter, Kisilak, Irving, & Huang, 2003; Thibos, Cheng, Phillips, & Collins, 2002) have attempted to measure monochromatic aberrations in younger chicks using Hartmann–Shack aberrometers (Liang et al., 1994).

In this study, we perform longitudinal measurements of refraction, axial length and monochromatic aberrations in occluded eyes and normal chick eyes during the first two weeks of development. The aims of the study were to investigate: (1) longitudinal changes of aberrations during normal emmetropization; (2) the effect of myopia development on ocular aberrations; (3) possible effects of natural aberrations on myopia development; (3) the differences in optical quality in myopic and emmetropic eyes; (4) longitudinal changes of aberrations in myopic eyes. Unlike myopia caused by lens treatment (where the lens + elongated eye tends to form an optically good system), form deprived eyes are subject to the continuous degradation produced by the diffuser. If we find that this treatment resulting in myopia also produces increased amounts of high order aberrations, we will favour the hypothesis that aberrations are a consequence, rather than a cause of myopia. In such a model, the enlargement of the eye (and subsequent modification of structural properties of the ocular components) would be the reason for the larger aberrations found in myopic eyes. Future experiments will aim at testing the hypothesis in the reversed direction, i.e., whether artificially induced aberrations may result in myopia development.

2. Methods

2.1. Subjects and experimental protocols

Ten White-Leghorn chicks were used in this experiment. All experimental protocols were approved by the Institutional Review Boards and followed the tenets of Helsinki. Seven chicks were monocularly treated and measured periodically. Another three, two untreated and one treated, were measured only on the last day, as control subjects (to discard possible interferences from the repeated measurements). All chicks were labelled with colour wires attached around their feet. Chicks were reared under fluorescent lighting (12 h/12 h light/dark cycle conditions) in a cage inside a controlled heated room (24–28 °C). They were allowed to eat and drink ad libitum. Adequate measures were taken to minimize pain or discomfort.

The seven non-control chicks were initially measured in their first day after hatching. This day was named "day 0". Days of age are therefore estimated adding one day to the measurement day. Immediately after the measurements, the right eye of each chick was occluded, and the non-occluded eye (left eye) was used as a reference. Occluders consisted of translucent diffusers which were manufactured with a sheet of plastic, moulded to obtain hemispherical translucent goggles (Frank Schaeffel, Personal communication). The occluders were attached with velcro rings glued to the feathers around the eye. They were only removed during measurements on days 0, 1, 4, 6, 8, 11, and 13. On these days we obtained measurements of refractive error, axial length and monochromatic aberrations in both eyes. An experimental session, including the three types of measurements, lasted typically 5 min per eye. All measurements were performed with the animals awake and under natural viewing conditions.

2.2. Refraction and ultrasound biometry

Refraction was measured using streak retinoscopy with trial lenses (Yinon, Rose, & Shapiro, 1980) in the horizontal meridian. Chicks were awake and unanaesthetized. We did not use cycloplegia nor lid-retractors.

An adapted ultrasound biometer (Allergan Humphrey Mod. 826) was used for axial length measurements. The probe was adapted to the chick eye's dimensions using a 10-mm tube filled in with water and covered with paraffin film, as described in the literature (Schaeffel & Howland, 1991). Measurements were conducted under topical anaesthesia (a drop of lidocaine 1%). Five data were obtained per condition.

2.3. Shack–Hartmann aberrometry

Aberrations were measured with a custom-built compact Hartmann–Shack (HS) wavefront sensor, which we built specifically to measure ocular aberrations in a chicken model. Fig. 1 shows a schematic diagram of our HS aberrometer. The light source is a 676 nm superluminescent diode (SLD) coupled to an optical fiber (Superlum, Moscow, Russia). The light was collimated to achieve a 2-mm almost parallel beam. When the

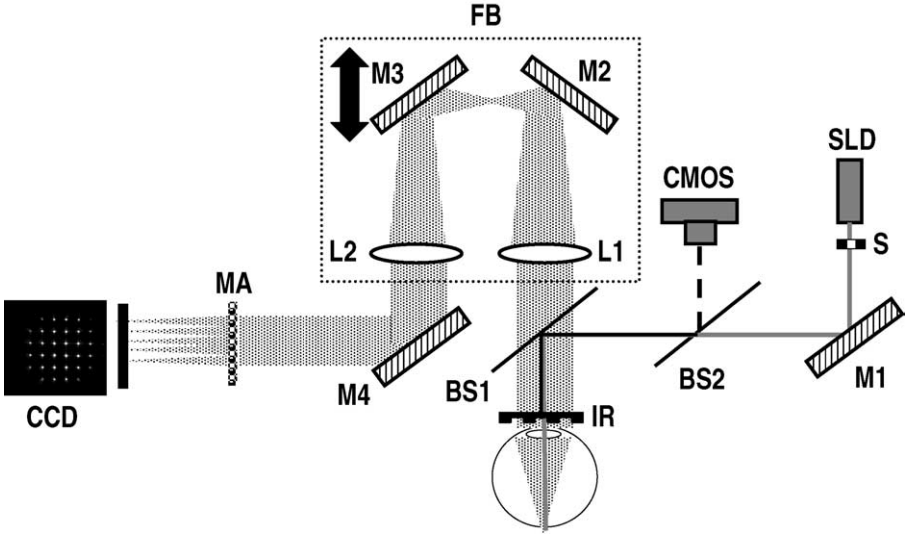

Fig. 1. Custom-built Hartmann–Shack wavefront sensor. The light source is a 676 nm superluminiscent diode (SLD). A shutter S blocks the light except during exposure and image capture. Mirror M1 and beam splitter BS1 direct the beam into the eye. A Badal focussing block (FB) formed by lenses L1 and L2 and mirrors M2 and M3 corrects defocus of the outgoing wavefront, which is reflected by mirror M4 and focused onto a high resolution cooled CCD camera through a microlens array (MA) placed on a pupil conjugate plane. The pupil monitoring channel consists on an LED ring placed in front of the eye, a beam splitter BS2 and a CMOS camera.

shutter (S) is opened, the beam is directed into the eye after reflection on mirror M1 and beam splitter BS1. The light reflected from the retina exits the eye and is projected by a focusing block (FB), mirror M4 and the microlens array MA onto a high resolution cooled CCD camera (Retiga 1300 Q imaging, Burnaby, Canada). The focusing block or Badal system is composed of two mirrors mounted on a translational stage, and two fixed lenses: L1, L2 ($f = 125$ mm), compensating for refractive errors (from -7 to $+9$ D) of the eye under test. Larger amounts of defocus are compensated with trial lenses. The microlenses array is placed on a pupil conjugate plane and consists of an array of 65×65 square microlenses with 24-mm focal length and 400-μm aperture. The pupil monitoring channel consists of an LED ring placed in front of the eye and a CMOS camera, allowing continuous viewing and pupil image recording. The HS image capture, pupil monitoring, and the electronic shutter are controlled by a computer using a custom-developed program written in Visual Basic (Microsoft Corporation, Redmond, Washington). The HS spot detection, centroiding algorithms and routines to obtain the wave aberration from the centroid shifts were written in Matlab (Mathworks, Natick, MA), and also developed specifically for this system.

The system was carefully aligned and calibrated before the measurements. Special care was taken to ensure a correct location of the microlens array (conjugate to the pupil) and CCD camera (at the microlens array focal plane) and to test for the linearity of the Badal system. The accuracy of the system was tested on spherical and cylindrical trial lenses as well as phase plates and artificial eyes with known high order aberrations. It was also tested against a laser ray tracing system (Llorente, Diaz-Santana, Lara-Saucedo, & Marcos, 2003; Marcos, Díaz-Santana, Llorente, & Dainty, 2002b), a well tested aberrometer developed in our laboratory, obtaining similar results (within the experimental error).

The entire system is mounted on an x–y translational stage. The chick sits on an elevating platform mounted in front of the system, which was moved to ensure correct centration and focusing of animal's pupil. The animal usually stayed quiet during the measurement, allowing us to capture several images per eye. The number of spots captured per image was related to the pupil size. We estimated pupil diameter as the distance between the two most separated spots in a HS image. We found that pupil increased with age, from 1.8/1.72 mm (treated/untreated eyes) on day 0 to 2.3/2.9 mm. on day 13, on average. Fig. 2 shows three typical examples of HS images from chick eyes, to allow comparison with typical retinal images in the human eye and other species'.

The HS images were processed using routines written in Matlab. The lateral deviations of each spot from their ideal positions (for a non-aberrated eye) were estimated. The centroids corresponding to each retinal spot were estimated by fitting the intensity profiles to a gaussian function. This procedure also allowed automatic rejection of spurious reflections (which were uncommon). Zernike coefficients were obtained by modal fitting of the lateral deviations to the derivatives Zernike polynomial expansions up to the 5th order. We obtained a maximum of 20 images per condition and selected the best five. Presence of artefact reflections, limited number of spots or low intensity were used as rejection criteria. Data were processed for the maximum pupil diameter (ranging from 1.5 to 3.26 mm). However, for comparative purposes across eyes and days the minimum pupil diameter of 1.5 mm was used. The optical quality of the eye was assessed in terms of individual Zernike terms or orders and root-mean-square wavefront error (RMS). Modulation transfer functions (MTF) and point spread functions (PSFs) were also obtained from the wave aberrations. Strehl ratio, estimated as the volume under the MTF normalized to the diffraction-limited eye, was also used as an optical quality metric.

2.4. Statistical analysis

We used an univariate ANOVA to test the changes with time and global differences between treated and non-treated eyes, using the eye a fixed factor, chick as a random factor and day as a covariate factor, with eye × time interactions. An unpaired t test was used to test differences between treated and untreated eyes on individual days.

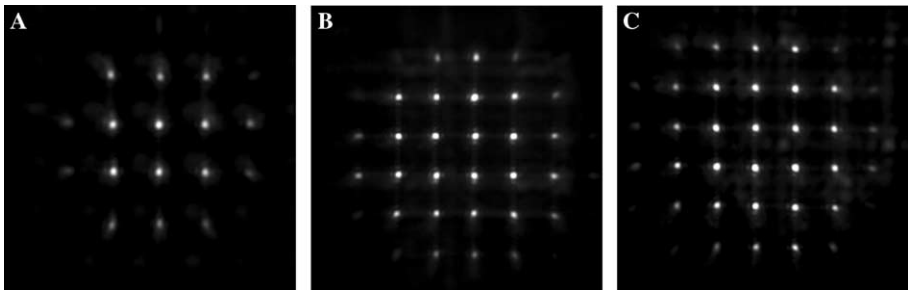

Fig. 2. Examples of Hartmann–Shack images on chick eyes. Images of the chick #5 at: (A) day 0: before occlusion, (B) day 13: treated eye, and (C) day 13: untreated eye.

3. Results

3.1. Ultrasound biometry and refraction

Fig. 3A shows axial length as a function of age in both eyes of the monolaterally treated chicks. Data from all chicks are shown, with open symbols representing non occluded eyes and solid symbols representing the corresponding occluded contralateral eye. While both eyes elongate during the first weeks of life ($p < 0.0001$; univariate ANOVA), the occluded eyes grow at a faster rate, and are significantly longer than the non occluded eyes ($p < 0.0001$; univariate ANOVA). The mean growth rate is 0.05 mm/day in non-occluded eyes and 0.12 mm/day in occluded eyes. Axial length increased from 7.2 ± 0.4 mm in non-occluded eyes and 7.1 ± 0.1 mm in occluded-eyes on average on day 0 (prior to treatment) to 7.9 ± 0.2 mm in non-occluded eyes and 8.8 ± 0.3 mm in occluded eyes on day 13. Control measurements on eyes that were left untreated or monolaterally occluded, but only measured on day 13 (to ensure that measurements did not interfere with normal emmetropization or the treatment) revealed similar results. We found 0.2 ± 0.3 mm interocular axial length difference in two chicks without any treatment, whereas 1.5 ± 0.1 mm interocular axial length difference between the occluded and non-occluded eyes of a third non-occluded chick. Fig. 3B shows refraction as a function of age in both eyes of the monolaterally occluded chick eyes. Each symbol represents a chick (open symbols are non occluded eyes and solid symbols occluded eyes). According to refraction, all eyes were hyperopic on day 0 prior to treatment (OD: $+4.5 \pm 1.2$ D; OS: $+4.1 \pm 1.6$ D) but differences between eyes are statistically significant ($p < 0.0001$; univariate ANOVA) from day 1 ($p = 0.01$; unpaired t test). Refraction tends gradually toward less hyperopic (non-occluded eyes) or more myopic values (occluded eyes). Refraction changes at a rate of 0.21 D/day in the non-occluded eye and 1.53 D/day in the occluded eye. By day 13, the non-occluded eyes show an average refraction of $+0.9 \pm 0.7$ D while occluded eyes show an average refraction of -16 ± 3 D. As we found for axial length, the non-occluded chicks show the same trends in refraction as

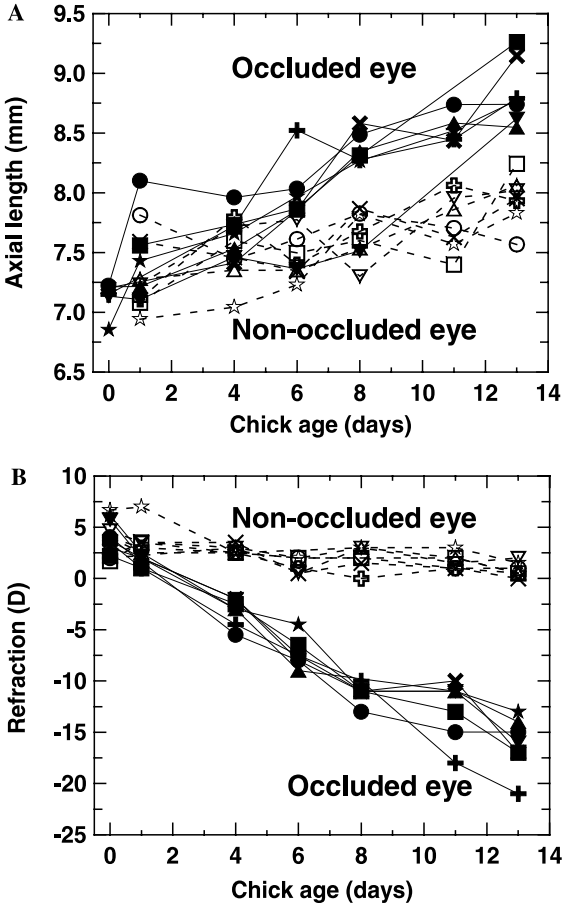

Fig. 3. (A) Axial length as a function of age. (B) Refraction as a function of age. Each symbol type corresponds to a different chick. Open symbols and dotted lines correspond to non-occluded eyes and solid symbols and solid lines correspond to occluded eyes.

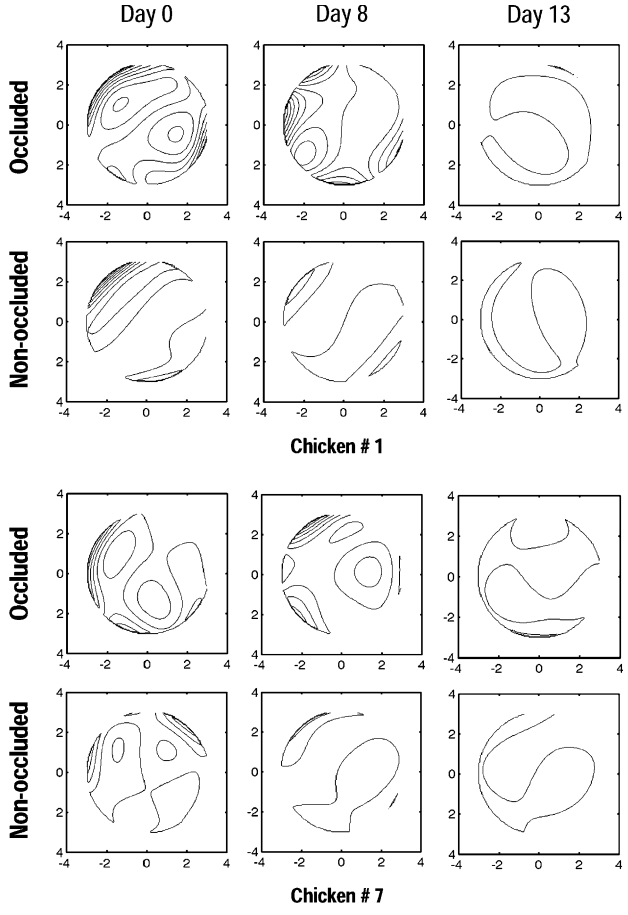

Fig. 4. Wave aberration patterns for chick #1 (represented by circles in Fig. 2) and #7 (crosses) on days 0, 8, and 13. Data are for 3rd and higher order aberrations and 1.5 mm. Contours are plotted every 0.2 μm.

the chicks that were measured repeatedly throughout the study: non-occluded chicks showed 0.5 and 1.50 D difference between eyes, respectively, while monolaterally occluded control chicks showed an interocular difference of 18 D.

3.2. Optical aberrations

Fig. 4 shows wave aberration patterns for days 0, 8 and 13 on chicks #1 and #7 corresponding to the eyes labelled with circles and crosses, respectively in Fig. 3. Data are for 3rd and higher order aberrations and 1.5-mm pupil diameters. In both occluded and non-occluded eyes, aberrations decrease with age and non-occluded eyes show lower amounts of aberrations than the occluded eyes. These trends are common in all eyes. Fig. 5 shows longitudinal mean changes of 3rd and higher order RMS (A), 3rd order RMS only, and spherical aberration (B and C). For comparison, all RMSs have been computed for the same pupil diameter (1.5 mm). RMS decreases gradually and significantly with age ($p < 0.0001$; univariate ANOVA), and this happens in both non-occluded and occluded eyes, with differences being statistically significant between both groups ($p = 0.01$). Prior to occlusion (day 0), RMS is similar in both eyes ($p = 0.8$) but RMS is significantly higher in the occluded eyes on days 8, 11 and 13 ($p = 0.005$, $p = 0.001$ and $p = 0.03$, respectively; unpaired t test). From days 8 to 13, both eyes follow an approximately parallel decrease in RMS, with occluded eyes showing higher RMS values in all cases. We found larger inter-subject variability in younger (days 0–4) than older chicks (days 6–13) with 0.05 vs. 0.02 μm average standard deviations across individuals, respectively. Measurements are also noisier in younger than older chicks: 0.2 and 0.08- μm standard deviations, respectively for repeated measurements. Third and higher order RMS decreased from $0.12 \pm 0.05/0.11 \pm 0.03$ μm at day 0 to $0.03 \pm 0.01/0.06 \pm 0.03$ μm at day 13 for non-occluded/occluded eyes. Third order RMS decreased from $0.09 \pm 0.04/0.08 \pm 0.02$ to $0.02 \pm 0.01/0.04 \pm 0.02$ μm.

Average changes of spherical aberration with age are shown in Fig. 5 (in terms of RMS in C and 4th order spherical aberration Zernike coefficient in D). In the first 4 days the tendency is irregular in both non-occluded and occluded eyes, and tends to stabilize after day 6. Older non occluded eyes show spherical aberration very close to 0, while occluded eyes show slightly negative spherical aberration. On day 13, spherical aberration is practically 0 in both groups: -0.001 ± 0.006 and $+0.002 \pm 0.009$ μm in non-occluded and occluded eyes,

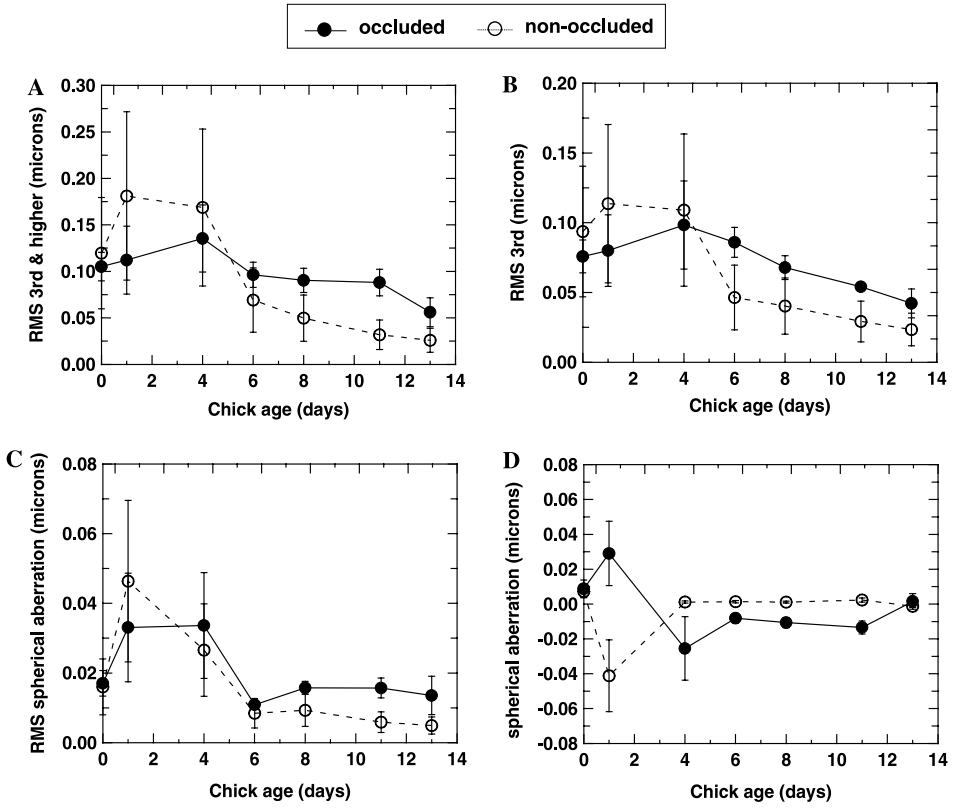

Fig. 5. Mean 3rd and higher order RMS (A), 3rd order RMS (B), spherical aberration RMS (C), and 4th order spherical aberration Zernike coefficient (D) as a function of age, averaged across all chicks. Pupil diameter: 1.5 mm. Open symbols and dotted lines correspond to non-occluded eyes and solid symbols and solid lines correspond to occluded eyes. Error bars stand for standard deviations.

respectively. Differences with age are not statistically significant ($p = 0.4$; univariate ANOVA), nor the differences between treated and non-treated eyes ($p = 0.1$).

3.3. Modulation transfer function

Prior to treatment both eyes show similar MTFs. The MTFs increase gradually with time both in the occluded and non-occluded eye, approaching the diffraction-limit MTF by the end of the experiment. After day 6, MTFs of non-occluded eyes tend to be higher than in the occluded eyes. The MTF in non-occluded eyes is higher than in occluded eyes in all chicks except one on day 6, in all chicks on day 8 and 11 and in all but two chicks on day 13. Fig. 6 represents MTF ratios (non-occluded/occluded eye) for all chicks for day 8. Values are greater than 1 for all spatial frequencies and subjects, indicating better optical quality in non-occluded eyes. MTF ratios (averaged across spatial frequencies) range between 1.08 for chick #5 and 2.02 for chick #1. Differences between the non-occluded and occluded eye tends to increase with spatial frequency and in some cases peak at mid-spatial frequencies. Fig. 7A shows modulation transfer as a function of age for two different spatial frequencies, 1.5 and 7 c/deg, which seem to be relevant for the chick's visual system (Troilo & Wallman, 1991). After day 4, the occluded eye tends to show lower modulation than the non-occluded eye for 1.5 c/deg, but differences are in general not statistically significant. However, for 7 c/deg differences between occluded and non-occluded eyes are globally significant ($p = 0.01$; univariate ANOVA). Fig. 7B shows mean Strehl ratio (as a global image quality metric) as a function of age, showing consistent improvement of optical quality with age in both eyes. Strehl ratio increases from 0.57 ± 0.10 on day 0 to 0.95 ± 0.05 on day 13 in non-occluded eyes, and from 0.56 ± 0.14 to 0.79 ± 0.15 for the occluded eyes. Changes in Strehl ratio with age are significant ($p < 0.0001$; univariate ANOVA) as well as the global differences between non-occluded and occluded eyes ($p < 0.0001$; univariate ANOVA). On days 8 and 11 Strehl ratios are significantly better in the non-occluded than in the occluded eye ($p = 0.006$ and $p = 0.05$, respectively; unpaired t test). Differences are reduced on day 13 ($p = 0.06$), with both eyes being practically diffraction-limited at the end of the experiment (according to the Raileigh criterion) for 1.5 mm pupils.

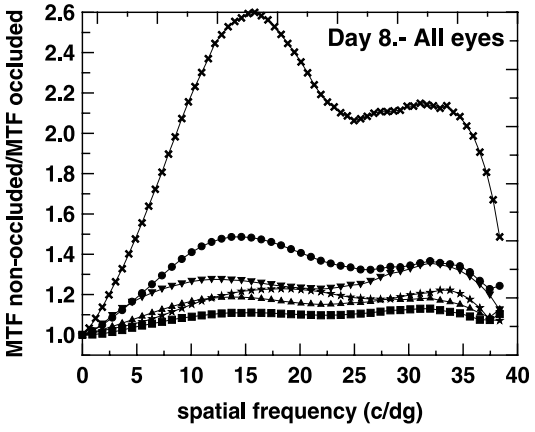

Fig. 6. MTF ratios non-occluded/occluded eye for all chicks on day 8. MTF ratios (averaged across spatial frequencies). All data are for pupil diameter of 1.5 mm. Each symbol type corresponds to a different chick.

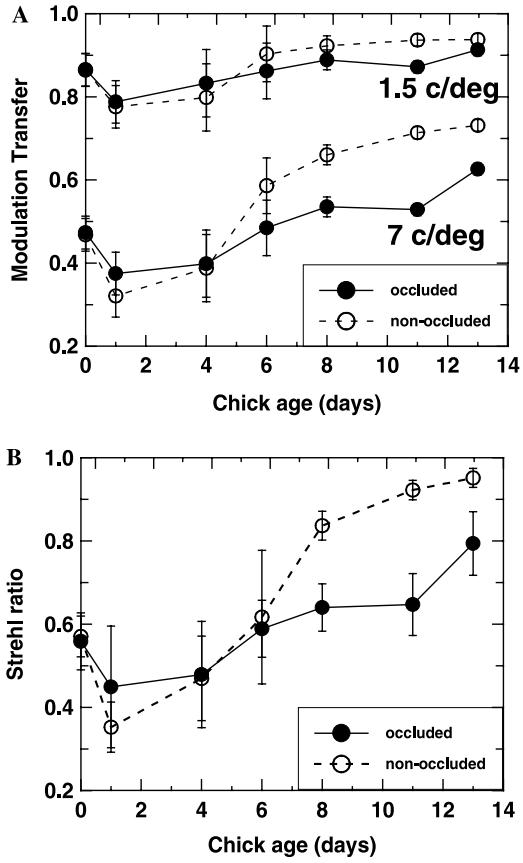

Fig. 7. (A) Mean modulation transfer as function of age (averaged across all chicks) for two different spatial frequencies, 1.5 and 7 c/deg. Open symbols and dotted lines correspond to non-occluded eyes and solid symbols and solid lines correspond to occluded eyes. Error bars stand for standard deviations. (B) Mean Strehl ratio as a function of age. Open symbols and dotted lines correspond to non-occluded eyes and solid symbols and solid lines correspond to occluded eyes. Error bars stand for standard deviations.

4. Discussion

4.1. Comparison with previous studies in experimental models

Our method of myopia induction in chicks by depriving forms has been widely used and studied. Normal eyes in our study developed as reported in the literature (starting moderately hyperopic, with a progressive tendency

toward emmetropia). Our refraction and axial length changes in occluded eyes are consistent with results from previous studies in White-Leghorn chicks, although for similar treatment periods our average myopia outcomes were slightly lower. A previous study (Guggenheim, Erichsen, Hocking, Wright, & Black, 2002) found in a similar experiment with restricted vision in one eye and normal vision in the contralateral eye, interocular differences of 1.4 ± 0.4 mm in axial length and 26.4 ± 7 D in refraction, after 2 weeks of treatment. Our results on day 13 showed interocular differences of 0.9 ± 0.4 mm and 17 ± 3 D, respectively. Another study (Schmid & Wildsoet, 1997) using constant form deprivation with diffusers reported interocular differences of 0.49 ± 0.10 and 0.82 ± 0.20 mm in axial length, and -12 ± 3 and -19 ± 6 D in refraction, on days 5 and 10, respectively. We obtained interocular differences of 0.5 ± 0.5 and 0.80 ± 0.3 mm in axial length and -10 ± 2 and -14.8 ± 3 D in refraction, on days 6 and 11, respectively. While the outcomes are similar, we obtained slightly lower values. One reason for the differences between studies could have been the amount of diffusion produced by the occluder, since correlations between the amount of myopia induced and the density of the diffuser material have been demonstrated (Bartmann & Schaeffel, 1994). Another potential factor contributing to lower myopia outcomes could have been the fact that we took out the occluders for brief time periods while we were taking the measurements, and given that additional measurements (Hartmann–Shack aberrations) required longer measurement times, chicks may have been exposed to longer periods of "normal viewing" than in previous studies. It has been shown (Schmid & Wildsoet, 1997) that, if the treatment is interrupted with 20 min of "visual stimulation" each day, form-deprivation myopia is significantly reduced. However our control chick (monolaterally treated, but not measured during intermediate days) developed an interocular refraction difference of 18 D, similar to the average refraction on day 13 that we found on the occluded eyes that participated in all measurements. Differences cannot be attributed to the fact that all chicks in our experiment were males since it has been shown (Guggenheim et al., 2002) that there is no sex-related difference in refraction data following form-deprivation, and if anything, there is a slightly higher elongation ($\sim$0.2 mm) in males than females in three strains of chickens, included White-Leghorn.

To our knowledge, there are only two studies, reported in scientific meetings, which measured optical aberrations in chick eyes (Campbell et al., 2003; Kisilak, Campbell, Irving, & Hunter, 2002; Thibos et al., 2002). A previous study (Coletta et al., 2003) measured the modulation transfer function (MTF) using a double pass technique in older chickens' eyes, both normal and myopic after different treatments. Aberrometry allows individual assessment of individual Zernike terms, as well as estimates of point spread functions (PSF) and modulation transfer function for any pupil size and defocus, while the double-pass technique only allows measurement of MTF for the pupil size and focus correction of the measurement.

Thibos et al. (2002) measured higher-order optical aberrations in normal chicks during the first week of life with a HS aberrometer. When normalized by pupil area, the equivalent defocus of all the Zernike modes decreased slightly with age, a tendency in agreement with our finding of the increasing optical quality with age (in our case for a constant pupil diameter). However, they concluded that the optical quality during the first week of life in the chick eye is significantly worse than in human adult eyes, while we found good optical quality in chicks (for 1.5 mm pupils), and close to diffraction-limit by day 13 in non-treated eyes. Coletta et al. found relatively good optical quality in chick eyes, although worse than in human eyes. However, their data are for older chicks (from 3 to 6 weeks old) and larger pupils (4.50-mm mean pupil diameter) than in Thibos' or the current study. In any case, our results support Coletta et al.'s conclusions that optical quality is not limiting spatial resolution in chicks, since the MTF's cut-off frequencies are well above reported chicks spatial acuity: 1.5 c/deg from behavioural studies (Over & Moore, 1981) or up to 8.6 c/deg from optokinetic nystagmus responses (Schmid & Wildsoet, 1998). Campbell et al. (2003) also found an improvement with age of the optical quality of young normal chicks, for 1.6-mm pupils. All reports show trends of decreased optical quality in myopic eyes, regardless the method of myopia induction. Coletta et al. (2003) found that myopic eyes had poorer optical quality than normal chicks. Unlike our study, where we induced myopia with diffusers, Campbell et al. induced myopia in chicks with -15 D lenses. They found that average optical quality (for 1.6 mm) did not change between days 0 and 7, unlike control eyes that experienced a decrease in the amount of aberrations. For higher order aberrations alone, goggled eyes had significantly worse optical quality at day 7 than controls. While we also found significantly less aberrations in control eyes than in treated eyes, we found that higher order aberrations decrease in both normal and treated eyes. However, it should be noted that in our experiment, the most significant differences occur after day 8, and trends are observed when extending the experiment for at least five more days.

Similar tendencies were found recently in mammal models (Ramamirtham et al., 2004). These authors found that manipulation of visual experience with diffusers or spectacle lenses in young Rhesus monkeys resulted in greater amounts of ocular aberrations, with no significant differences in the magnitude or pattern of higher order aberrations between the control and treated groups before treatment and significant RMS differences (0.09 μm) by the end of the treatment period.

4.2. An emmetropization of the optical aberrations?

We found that aberrations tend to decrease during development in chicks. This was also found by Thibos et al. (2002) and Campbell et al. (2003) in normal chick eyes. While working with chicks allows longitudinal measurements, some cross-sectional measurements in the literature are suggestive that a similar tendency is found in humans. Human results reported by Brunette, Bueno, Parent, Hamam, and Simonet (2003) showed that optical aberrations decrease during development. These authors measured optical aberrations in subjects ranging from 5.7 to 82.3 years and found that the average optical quality in early childhood was significantly worse that in the advanced age, with aberrations decreasing during childhood and adolescence. It is well known that the optical aberrations of the crystalline lens (showing negative spherical aberration) partially compensate corneal aberrations (showing positive spherical aberration) in the normal young human eye, and that this compensation gets disrupted later in age (Calver, Cox, & Elliott, 1999; Artal, Berrio, Guirao, & Piers, 2002; Mclellan, Marcos, & Burns, 2001). Brunette et al.'s cross-sectional data, as well as the mentioned longitudinal data in chicks, may suggest that the optimal performance found in young adults is reached after an optimization process that takes place during development. Other authors (Wang, Tondel, & Candy, 2004), however found that the optical quality was as good in infants (5–7 weeks) as in young adults (younger than 40 years), with no significant difference in the levels of 3rd order monochromatic aberrations, and only a higher tendency in infants to show negative spherical aberration with adults eyes tending to show positive spherical aberration.

Aberration balance between optical components, and even more a potential improvement of the optical quality of the eye during development, may lead to consider an active process for the development of optical components. If an active visually guided process tunes the eye length to the power of the optical component, one may think of a similar system adjusting the optical and geometrical properties of the optical components to reduce high order aberrations and produce optimal image quality. Our results do not support such as system, or at least this process being visually guided. We found that the improvement of the optical quality with age occurs even in the eye occluded with diffusers, subject to dramatic image quality degradation. While a lens treatment may have provided a different approach to answering this question, excluding any visual feedback with the diffusers suggests that the tuning of optical aberrations of ocular components is likely the result of a pre-programmed process or just geometrical scaling but it does not seem to rely on visual experience to occur, at least to a great extent. These findings are in good agreement with a scaling model recently proposed by Howland (2005). This model, based on reported data of corneal curvature increase in White-Legorn chicks during the first week of life, shows that aberrations measured in a growing eye at a constant pupil size decrease with time. A more elaborate model, including geometrical properties of the cornea and crystalline lens of the developing myopic and normal eye would be necessary to assess if scaling accounts for the all the decrease in aberrations and to explain the differences between both eyes.

4.3. Optical aberrations and emmetropization

We found higher amounts of optical aberrations in myopic eyes than in the normal control eyes after six days of treatment. While the differences are significant, the amount of blur produced by aberrations is minimal compared to the optical degradation produced by the diffuser or the developed refractive error. By day 13 even myopic eyes are close to diffraction-limited. These experiments shed light on possible relationships between aberrations and myopia development. There are several cross-sectional studies in humans reporting optical aberrations as a function of refractive error (Carkeet, Luo, Tong, Saw, & Tan, 2002; Cheng, Bradley, Hong, & Thibos, 2003; Collins, Wildsoet, & Atchison, 1995; Llorente, Barbero, Cano, Dorronsoro, & Marcos, 2004; Marcos, Barbero, & Llorente, 2002a; Paquin et al., 2002). Most studies found higher amounts of aberrations as myopia increased (Atchison et al., 1995; Coletta et al., 2003). Several studies only found a significant correlation for high myopes, and third order aberrations, but not spherical aberration (Marcos et al., 2002a). One study (Carkeet et al., 2002) did not found correlations between refractive error and high order aberrations (for myopic Singaporean children, <3 D), and another study (Cheng et al., 2003) on 200 normal human eyes failed to find correlations between high order aberrations and refractive errors (from $+5.00$ to -10.00). In the present study, in chicks, interocular statistically significant differences in the amount of higher order aberrations only appear for amounts of myopia beyond -7.3 D. The fact that increased amounts of aberrations are found in higher myopes may lead to the hypothesis that aberrations may be a cause for myopia. Suggestive evidence of this hypothesis has been presented in the introduction. Longitudinal measurements allow light to be shed into the question whether higher aberrations are a cause or an effect of myopia development. Our experiment clearly favours the hypothesis that aberrations are a consequence of the structural changes occurring in the excessively elongated eye: (1) We did not find that eyes with higher amounts of aberrations at birth emmetropized less efficiently; (2) The retinal image degradation imposed by diffusers induces myopia in the treated eyes. Unlike a potential treatment with lenses, where eye/lens system can project good optical quality

images on the retina, a treatment with diffusers allows no visual feedback. Treated eyes turned out to be more aberrated most likely as a result of the treatment, but it is unlikely that the increased aberrations may have played any role at all in the development of myopia. (3) While aberrations are significantly higher in myopic eyes than in the normal eyes, the retinal image degradation induced is negligible compared to the degradation imposed by the diffuser and the induced defocus. While in the present experiment aberrations result from the myopia development, further experiments are planned to test the hypothesis in the reversed direction (aberrations as a potential cause for myopia development).

Acknowledgments

The authors acknowledge funding from Grant # BFM2002-02638, Ministerio de Ciencia y Tecnología, Spain and Grants #CAM08.7/004.1/2003, and GR/SAL/0387/2004, Consejería de Educacion Comunidad Autónoma de Madrid to Susana Marcos, and a CSIC-Unidades Asociadas predoctoral fellowship to Elena García de la Cera. The authors also acknowledge assistance from Lourdes Llorente and Jesús Merayo in different aspects of the study, and support from the Unidad Asociada IO-CSIC/IOBA-Universidad de Valladolid.

References

Artal, P., Berrio, E., Guirao, A., & Piers, P. (2002). Contribution of the cornea and internal surfaces to the change of ocular aberrations with age. *Journal of the Optical Society of America A Optics Image Science Vision, 19*, 137–143.

Atchison, D., Collins, M., Wildsoet, C., Christensen, J., & Waterworth, M. (1995). Measurement of monochromatic ocular aberrations of human eyes as a function of accommodation by the Howland aberroscope technique. *Vision Research, 35*, 313–323.

Bartmann, M., & Schaeffel, F. (1994). A simple mechanism for emmetropization without cues from accommodation or colour. *Vision Research, 34*(7), 873–876.

Brunette, I., Bueno, J. M., Parent, M., Hamam, H., & Simonet, P. (2003). Monochromatic aberrations as a function of age, from childhood to advanced age. *Investigative Ophthalmology & Visual Science, 44*(12), 5438–5446.

Buehren, T., Collins, M. J., & Carney, L. (2003). Corneal aberrations and reading. *Optometry and Vision Science, 80*(2), 159–166.

Calver, R., Cox, M., & Elliott, D. (1999). Effect of aging on the monochromatic aberrations of the human eye. *Journal of the Optical Society of America A, 16*, 2069–2078.

Campbell, M. C. W., Hunter, J. J., Kisilak, M. L., Irving, E. L., & Huang, L. (2003). Image quality on the retina of the chick eye during emmetropization: Goggled vs control eyes. *Investigative Ophthalmology & Visual Science, 44*, E-Abstract 1993.

Carkeet, A., Luo, H., Tong, L., Saw, S., & Tan, D. (2002). Refractive error and monochromatic aberrations in Singaporean children. *Vision Research, 42*(14), 1809–1824.

Cheng, X., Bradley, A., Hong, X., & Thibos, L. (2003). Relationship between refractive error and monochromatic aberrations of the eye. *Optometry and Vision Science, 80*, 43–49.

Coletta, N., Marcos, S., Wildsoet, C., & Troilo, D. R. (2003). Double-pass measurement of retinal image quality in the chicken eye. *Optometry and Vision Science, 80*, 50–57.

Collins, M. J., Wildsoet, C. F., & Atchison, D. A. (1995). Monochromatic aberrations and myopia. *Vision Research, 35*(9), 1157–1163.

Dorronsoro, C., Barbero, S., Llorente, L., & Marcos, S. (2003). On-eye measurement of optical performance of Rigid Gas Permeable contact lenses based on ocular and corneal aberrometry. *Optometry and Vision Science, 80*, 115–125.

Guggenheim, J. A., Erichsen, J. T., Hocking, P. M., Wright, N. F., & Black, R. (2002). Similar genetic susceptibility to form-deprivation myopia in three strains of chicken. *Vision Research, 42*(25), 2747–2756.

Hayes, B. P., Fitzke, F. W., Hodos, W., & Holden, A. L. (1986). A morphological analysis of experimental myopia in young chickens. *Investigative of Ophthalmology & Visual Science, 27*, 981–991.

He, J. C., Marcos, S., Webb, R. H., & Burns, S. A. (1998). Measurement of the wave-front aberration of the eye by a fast psychophysical procedure. *Journal of the Optical Society of America A, 15*, 2449–2456.

Howland, H. C. (2005). Allometry and scaling of wave aberration of eyes. *Vision Research, 45*(9), 1091–1093.

Kee, C. S., Marzani, D., & Wallman, J. (2001). Differences in time course and visual requirements of ocular responses to lenses and diffusers. *Investigative Ophthalmology & Visual Science, 42*(3), 575–583.

Khoo, C., Chong, J., & Rajan, U. (1999). A 3-year study on the effect of RGP contact lenses on myopic children. *Singapore Medical Journal, 40*, 230–237.

Kisilak, M. L., Campbell, M. C. W., Irving, E. L., & Hunter, J. J. (2002). Hartmann–Shack measurement of the monochromatic image quality in the chick eye during emmetropization. *Investigative Ophthalmology & Visual Science, 43*, E-Abstract 2924.

Liang, J., Grimm, B., Goelz, S., & Bille, J. F. (1994). Objective measurement of wave aberrations of the human eye with the use of a Hartmann–Shack wave-front sensor. *Journal of the Optical Society of America A, 11*, 1949–1957.

Llorente, L., Barbero, S., Cano, D., Dorronsoro, C., & Marcos, S. (2004). Myopic versus hyperopic eyes: Axial length, corneal shape and optical aberrations. http://journalofvision.org/4/4/5/. *Journal of Vision, 4*, 288.

Llorente, L., Diaz-Santana, L., Lara-Saucedo, D., & Marcos, S. (2003). Aberrations of the human eye in visible and near infrared illumination. *Optometry and Vision Science, 80*, 26–35.

Marcos, S., Barbero, S., & Llorente, L. (2002a). The sources of optical aberrations in myopic eyes. *2002 Annual Meeting Abstract and Program Planner accessed at* www.arvo.org. *Association for Research in Vision and Ophthalmology. Abstract 1510*.

Marcos, S., Díaz-Santana, L., Llorente, L., & Dainty, J. C. (2002b). Ocular aberrations with ray tracing and Shack–Hartmann wave-front sensors: Does polarization play a role? *Journal of the Optical Society of America A, 19*, 1063–1072.

Mclellan, J., Marcos, S., & Burns, S. (2001). Age-related changes in monochromatic wave aberrations in the human eye. *Investigative Ophthalmology & Visual Science*, 1390–1395.

Moreno-Barriuso, E., Marcos, S., Navarro, R., & Burns, S. A. (2001). Comparing laser ray tracing, spatially resolved refractometer and Hartmann–Shack sensor to measure the ocular wavefront aberration. *Optometry and Vision Science, 78*, 152–156.

Over, R., & Moore, D. (1981). Spatial acuity of the chicken. *Brain Research, 211*, 424–426.

Paquin, M. P., Hamam, H., & Simonet, P. (2002). Objective measurement of optical aberrations in myopic eyes. *Optometry and Vision Science, 79*(5), 285–291.

Perrigin, J., Perrigin, D., Quintero, S., & Grosvenor, T. (1990). Silicone-acrylate contact lenses for myopia control: 3-year results. *Optometry and Vision Science, 67*, 764–769.

Ramamirtham, T., Kee, C.-S., Qiao-Grider, Y., Hung, L.-F., Roorda, A., & Smith, E. L. (2004). Wave aberrations in rhesus monkeys with vision-induced ametropias. *Investigative Ophthalmology & Visual Science, 45*.

Schaeffel, F., Glasser, A., & Howland, H. (1988). Accommodation, refractive error and eye growth in chickens. *Vision Research, 28*, 639–657.

Schaeffel, F., & Howland, H. C. (1991). Properties of the feedback loops controlling eye growth and refractive state in the chicken. *Vision Research, 31*, 717–734.

Schmid, K. L., & Wildsoet, C. F. (1997). Contrast and spatial-frequency requirements for emmetropization in chicks. *Vision Research, 37*(15), 2011–2021.

Schmid, K. L., & Wildsoet, C. F. (1998). Assessment of visual acuity and contrast sensitivity in the chick using an optokinetic nystagmus paradigm. *Vision Research, 38*(17), 2629–2634.

Smith, E., III. (1998). Environmentally induced refractive errors in animals. *Myopia and Nearwork. Butterworth Heinemann Oxford*, 57–90.

Thibos, L. N., Cheng, X., Phillips, J., & Collins, A. (2002). Optical aberrations of chick eyes. *Investigative Ophthalmology & Visual Science, 43*, E-Abstract 180.

Troilo, D., Nickla, D. L., & Wildsoet, C. F. (2000). Form deprivation myopia in mature common marmosets (*Callithrix jacchus*). *Investigative Ophthalmology & Visual Science, 41*(8), 2043–2049.

Troilo, D., & Wallman, J. (1991). The regulation of eye growth and refractive state: An experimental study of emmetropization. *Vision Research, 31*, 1237–1250.

Wallman, J. (1993). Retinal control of eye growth and refraction. *Progress in Retinal Research, 12*, 133–153.

Wallman, J., & Adams, J. I. (1987). Developmental aspects of experimental myopia in chicks: Susceptibility, recovery and relation to emmetropization. *Vision Research, 27*, 1139–1163.

Wang, J., Tondel, G. M., & Candy, T. R. (2004). Higher-order monochromatic aberrations of human infant eyes. *Investigative Ophthalmology & Visual Science*, 45.

Wiesel, T. N., & Raviola, E. (1977). Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature, 266*, 66–68.

Wildsoet, C. F. (1997). Active emmetropization—evidence for its existence and ramifications for clinical practice. *Ophthalmic and Physiological Optics, 17*(4), 279–290.

Wilson, B. J., Decker, K. E., & Roorda, A. (2002). Monochromatic aberrations provide an odd-error cue to focus direction. *Journal of the Optical Society of America A—Optics Image Science and Vision, 19*(5), 833–839.

Yinon, U. (1984). Myopia induction in animals following alteration of visual input during development: A review. *Current Eye Research, 3*, 677–690.

Yinon, U., Rose, L., & Shapiro, A. (1980). Myopia in the eye of developing chicks following monocular and binocular lid closure. *Vision Research, 20*, 137–141.