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LONGITUDINAL CHROMATIC ABERRATION AND EMMETROPIZATION: RESULTS FROM THE CHICKEN EYE

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SUMMARY

1. Due to the chromatic dispersion of the ocular media, the focal length of the optics of the eye is about 3 diopters longer for red light than for blue light. Because emmetropization in the chicken (*Gallus domesticus*) does not require colour cues and operates properly in monochromatic light, one can, therefore, expect that chickens raised in red light become more myopic (with longer eyes) than chicks raised in short wavelength light. Prior to conducting this experiment, we matched the brightness of both light conditions by means of flicker electroretinograms such that equiluminance was obtained for the chickens.

2. Unexpectedly, refractive development was not different from controls in white light for either red or near-ultraviolet light.

3. We tested whether the visual mechanisms guiding refractive development were still sensitive to defocus under both illuminations by treating the chicks with spectacle lenses.

4. Similar to a previous experiment in white light, the growth of the eye in red light also changed such that it compensated for the imposed defocus. It failed to do so, however, in near-ultraviolet light.

5. A histological analysis of the sampling intervals for the ultraviolet receptor system revealed that its spatial resolving power was too low to detect the defocus imposed by the lenses, whereas the long wavelength receptors provided sufficiently good visual acuity.

6. The results show that, during emmetropization, the chicken eye elegantly bypasses the problem of multiple chromatic focal planes by having a low sensitivity to defocus in the blue end of the spectrum. Because the chromatic dispersion function is steep in the blue range but flat at the red end of the spectrum, the remaining chromatic defocus in the spectral range of high visual acuity is low and may match the depth of field of the eye.

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INTRODUCTION

From experiments in animal models of human refractive development, it became clear that the optical and geometrical precision of the eye during growth is also a result of visually guided feedback control (Raviola & Wiesel, 1985; Schaeffel, Glasser & Howland, 1988; Iuvone, Tigges, Stone, Lambert & Laties, 1991; Troilo & Wallman, 1991; Schaeffel & Howland, 1991) and not entirely predetermined by genetical factors (Sorsby, 1979). The focal length of the optics is actively matched to the axial length such that the retinal image quality remains optimal during growth. However, refractive errors can develop despite the presence of visual feedback loops for control of refractive state. Therefore, the way by which these feedback loops act has become a research topic of considerable interest. The basic questions are whether refractive errors can be explained as the result of inappropriately triggered feedback loops or failures in their components.

Because visual feedback control of eye growth is very precise (ultimately limited by the depth of focus of the eye), emmetropizing mechanisms have to account for chromatic aberration present in the eye. Longitudinal chromatic aberration results in a difference in location of the focal planes of about 3 diopters for light from both ends of the visible spectrum (Fincham, 1951; Mandelman & Sivak, 1983). Therefore, an emmetropizing mechanism can produce an optimal refractive state only for light from a small spectral range while some defocus remains for light from other parts of the spectrum.

We have studied the effects of longitudinal chromatic aberration on emmetropization in the domestic chicken. Currently, chickens provide the most powerful non-mammalian model to investigate mechanisms of emmetropization. Chicken eyes grow very rapidly (increasing in axial length by about 100 μm a day) and have an excellent optical quality. Accordingly, they are very sensitive to experimental manipulations of visual experience, and can develop 20 diopters of axial myopia within 1 week if the retinal image quality is degraded during this time. For the chicken, the spectrum of visible light ranges from 360 nm or less to 700 nm (van Norren, 1975; Wortel, Rugenbrink & Nuboer, 1987). Due to their large spectral range of sensitivity, chickens are particularly well suited for the study of how emmetropization works in the presence of chromatic aberration. Furthermore, it has been tested previously (Schaeffel & Howland, 1991; Wildsoet & Howland, 1991) that colour cues are not necessary to guide emmetropization in the chick. We raised chickens in near-ultraviolet light with a cut-off at about 400 nm, and in deep red light with a short wavelength cut-off at 665 nm. Between both light conditions, the chromatic defocus exceeds 3 diopters (Mandelman & Sivak, 1983) and is, therefore, well beyond the depth of focus of the eye (about 1 diopter in the chick). Therefore, we expected that, after the experiment, the eyes would differ in refractive state by approximately the amount of longitudinal chromatic aberration. Because this expected result was not obtained, we tested whether or not the emmetropizing mechanisms operated normally under both spectral conditions by raising the chicks with defocusing spectacle lenses.

METHODS

Animals

One-day-old chickens were provided by a local hatchery (Suppingen, FRG). Thirty-five male chicks were used in this study, and were housed in groups of seven. Animals were kept under a 12/12 h light/dark cycle (light: 8 a.m.–8 p.m.), with temperatures of 26 °C for the first 10 days and 21 °C for the rest of the time and a relative humidity of 55–65 %. All chickens had access to food *ad libitum*, whereas water was supplied individually several times a day during lens treatment, because wet leather hoods (see below) resulted in poor optical quality of the lenses. The experiments were performed under the supervision of the veterinarian of the University and the chicks were treated in accordance with the German laws for care of experimental animals (BGB1.I, p: 1319, 18-08-86).

Light conditions and housing of the chicks

Chickens were raised in a large Perspex hemisphere (diameter 0·9 m), the walls of which were covered with a highly reflectant white paint to ensure homogenous illumination. Light entered the hemisphere through a hole cut in the top. The light came from an optical bench in an adjacent room. Its spectral composition was controlled by different filters. Light from the long wavelength end of the spectrum was provided by a Schott cut-off filter RG665, and near-ultraviolet light by Schott filter UV-AL383, with maximal transmittance at 383 nm and a half-bandwidth of 24 nm. The filters were chosen based on the spectral sensitivity function of the chick to stimulate preferably the long wavelength cones and the ultraviolet-sensitive system, respectively (Fig. 1). Intensities were controlled by neutral density filters in the optical bench. They were kept low to ensure that only photoreceptors from the extreme ends of the spectrum were stimulated. The irradiance in the sphere was 48 mW m^{-2} for the red light and 66 mW m^{-2} for ultraviolet light. These values were previously determined by means of isoluminant flicker electroretinograms and were found to provide similar relative brightnesses for the chicken eye. The spectral composition of the light in the hemisphere was carefully analysed with a spectroscope but no sideband transmission of the filters was detected.

Techniques

Leather hoods and lenses

Lens experiments were performed on three groups of seven chickens, as described by Schaeffel *et al.* (1988). During the light cycle, chickens wore small leather hoods of individual sizes to which ophthalmic lenses could be attached by Velcro fasteners. Lenses were frequently removed for a few seconds for cleaning to assure good optical quality at all times. Both eyes were treated with lenses of equal power but different sign (+4/−4 diopters). The treatment is justified because accommodation and pupillary reaction are asymmetrical in the chicken (Schaeffel *et al.* 1986), and because growth in both eyes can compensate for the defocus imposed by the lenses independently as well (Schaeffel *et al.* 1988). The animals were removed from the hemisphere every second day to measure refractive state and axial eye length. Because the room light was kept dim and the animals did not wear their lenses during this time, their exposure to white light during this period could have no effect on the experiment.

Measurements of the refractive state, corneal radius of curvature and axial eye length

The refractive development of the uncyclopleged eyes was measured by infra-red photoretinoscopy (Schaeffel, Farkas & Howland, 1987; Schaeffel & Howland, 1991). Infra-red photoretinoscopy is an eccentric photorefraction which uses high output infra-red light-emitting diodes (emission peak at 890 nm) as light sources. Because the light is invisible, the pupils remained large which improved the precision of the refractions.

Because low refractive errors cannot be distinguished from possible differences in the resting tonus of accommodation, it is necessary to confirm that the morphology of the eyes had changed in response to treatment with ophthalmic lenses. Morphological data were obtained from A-scan ultrasound measurements, and by measuring the radius of curvature of the corneal surface with infra-red photokeratometry (Schaeffel & Howland, 1987). The technique is very precise with a

standard deviation of less than 0·04 mm, which corresponds to about 1 diopter in the chicken. Infra-red photokeratometry was used only once at the end of each lens experiment to determine the intraocular differences in corneal radius of curvature. The axial length of the eyes was measured every second day by an A-scan ultrasound machine ('Echorule', 3M, Vision care). The transducer S/N OA476, originally designed to measure the human eye, was modified as described by Schaeffel & Howland (1991). For measurements, the eyes were locally anaesthetized with one to two drops of 2% Xylocaine solution (20 mg lidocaine hydrochloride (ml water)$^{-1}$). Precision of measurement was about 0·06 mm for thickness and position of the ocular lens and for axial eye length, as determined from the standard deviations of four to six repeated measurements. A change in axial length of 0·06 mm results in about 1 diopter of defocus in the age group of chickens used in this study (Schaeffel & Howland, 1988).

Electroretinograms

Recording apparatus. This consisted of a peripheral processor (PDP 11/23) used to control stimulus parameters and to store the digitized responses, and a dual-beam optical bench to provide the light stimuli. The light intensity in both beams, the test and the adaptation beam, was controlled by neutral density wedges with a precision of 0·01 log units over a range of 8 log units. The wedges were driven by stepping motors. A monochromator Jobin Yvon H10 was used to produce monochromatic light in the test beam (half-bandwidth 16 nm). Electroretinograms were filtered (bandpass, 0·01–400 Hz) and amplified by a factor of 2000. Single responses were displayed on-line on an oscilloscope; the digitized averaged responses were displayed on a second oscilloscope and stored in the computer for later analysis. The recording apparatus and procedures are described in more detail elsewhere (Schaeffel, Rohrer, Lemmer & Zrenner, 1991).

Procedure. Animals (body weight 160–240 g, age 21–45 days) were anaesthetized by ether and received subsequently an intraperitoneal injection of chloropent (0·5 ml (100 g body weight)$^{-1}$; Wallman & Adams, 1987). The chickens were placed in a hand-made holder in which their heads were fixed by a metal ring in the neck. No pressure points were necessary for stable recordings. The pupil of the left eye was dilated with vecuronium bromide (Pettigrew, Wallman & Wildsoet, 1990) and a specially designed miniature contact lens electrode was inserted (Henkes' type, Medical Workshop, Groningen, The Netherlands). The reference electrode was a subcutaneous stainless-steel needle, placed into the skin above the contralateral eye. A silver wire fixed to the electrode holder served as ground. The stimuli were presented in a Ganzfeld mode.

Spectral sensitivity was analysed without background light and without previous dark adaptation during day-time between 1 p.m. and 6 p.m. Light flashes with a duration of 100 ms and an interstimulus time of 100 ms were used, to allow both rods and cones to contribute to the electroretinogram (ERG) response (Fain & Dowling, 1973). However, it was found that, during day-time, the chicken ERG was a pure cone response, and that rod responses could not be isolated even under conditions of dark adaptation (Schaeffel *et al.* 1991). Therefore, the role of the rods was neglected in the present study. It was assured, by repeating reference measurements, that the adaptational level was stable throughout the data collection. Twenty sweeps were averaged and the a- to b-wave amplitude was determined. Response *versus* intensity functions were obtained for each stimulus wavelength in steps of 0·5 log units. Energy data were subsequently converted to log quanta s^{-1} μm^{-2}. The spectral sensitivity function was determined by a criterion method. We also confirmed that the ocular media transmitted near-ultraviolet light with no attenuation. This was similar to results presented by Govardowskii & Zueva (1977).

RESULTS

Spectral sensitivity of the chickens

The photopic spectral sensitivity function of the chickens used in the current study is shown in Fig. 1. The data points are averages of measurements in five chickens and are plotted with ± 1 standard deviation. Figure 1 also shows the spectral transmittance of the filters used to raise the chicks in light at two ends of their visible spectrum. Chickens show a broad sensitivity maximum between 520 and 620 nm and a second maximum in the near-ultraviolet range with a lower sensitivity of about

1·5 log units. The short wavelength sensitivity is in agreement with the ultraviolet sensitivity reported for many birds between 370 and 390 nm (van Norren, 1975; Delius & Emmerton, 1979; Goldsmith, 1980; Chen, Collins & Goldsmith, 1984; Wortel *et al.* 1987). Because the two peaks are separated by a trough of low

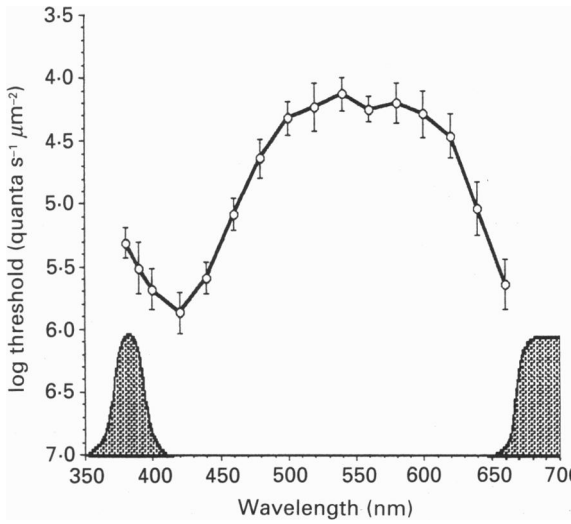

Fig. 1. Spectral sensitivity function of the chickens. Averages from five chickens are shown. Error bars denote standard deviations. Spectral sensitivity was determined by measuring response *vs.* intensity curves in steps of 0·25 log units for wavelengths from 370 to 680 nm, in steps of 20 nm. On the ordinate, log quanta s^{-1} μm^{-2} are plotted which were necessary to elicit a 60 μV criterion response. The figure also shows the transmittance of the filters used to provide near-ultraviolet and red light, respectively.

sensitivity at 420 nm, for low light intensities the ultraviolet-sensitive system can be stimulated separately by using the filter UVAL 383 (Fig. 1). The remaining four cone types (P506, P533, P569 and P606; Bowmaker & Knowles, 1977; Jane & Bowmaker, 1988) merge to a common peak and cannot be separately stimulated. However, if filter OG665 is used, mainly P606 and P659 are stimulated.

Effects of rearing in red or blue light on refractive development

To test whether chromatic defocus can affect refractive development of the eye, during rearing of the chickens we selectively stimulated the photoreceptors from the extreme ends of the spectrum. As shown by Mandelman & Sivak (1983), the focal planes differ by more than 2 diopters for these two illumination conditions and one would then expect that chicks become more myopic in red light than in blue light. A group of seven chickens was raised in deep red light (cut-off filter RG665) and a second group in near-ultraviolet light (filter UVAL 383). The results are shown in Fig. 2, with refractions plotted as a function of age. All eyes remained slightly hyperopic, with no significant difference from data from animals raised in white light (d.f. = 26, $t = 1{\cdot}71$, $P < 0{\cdot}1$, n.s., t test; Wallman *et al.* 1981; Schaeffel & Howland, 1988), and also no difference from each other. Both groups started out at $+1{\cdot}79 \pm 0{\cdot}65$ diopters

and reached a refractive state of $+1{\cdot}65 \pm 0{\cdot}69$ diopters at the end of the experiment (d.f. = 26, $t = 0{\cdot}55$, $P > 0{\cdot}5$, n.s., t test). As in previous studies (Schaeffel & Howland, 1988), the small amount of hyperopia inherent in the measurements is attributed to the 'small eye artifact of retinoscopy' (Glickstein & Millodot, 1970).

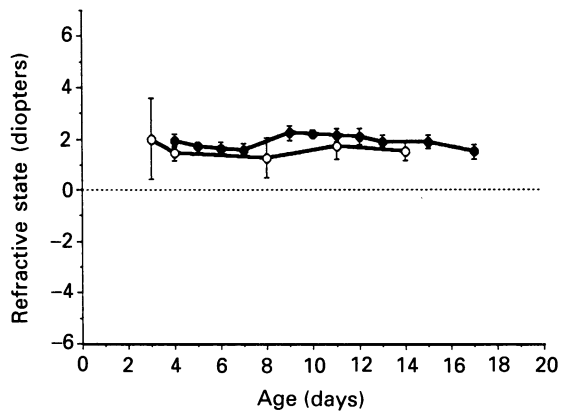

Fig. 2. Development of refractive state in two groups of chickens ($n = 7$) raised under near-ultraviolet light (●) and in deep red light (○) plotted as a function of age. Error bars denote standard deviations from measurements in fourteen eyes. The brightness of the illumination for both rearing conditions was matched previously by means of isoluminant flicker ERGs. Despite the difference in location of the focal planes in both experiments due to longitudinal chromatic aberration, refractive development was indistinguishable and also not different from control groups raised in white light.

Because chromatic defocus does not affect refractive development, it can be concluded that the visual mechanisms for control of eye growth and refractive state can emmetropize the eye for the mid-wavelength range despite the presence of longitudinal chromatic aberration.

Experiments with defocusing lenses

Control experiment with lenses in white light

Experiments with defocusing lenses permit one to study active emmetropization under closed-loop conditions. To allow for a comparison of the magnitude of possible effects of the lenses on refractive development, we raised chicks with lenses in the hemisphere illuminated by white light. Five different parameters were analysed: refractive status of the eye, axial length, anterior chamber depth, lens thickness and corneal radius of curvature. The results are shown in Fig. 3*A* and *B*. For both types of lenses, mean values from seven equally treated eyes and their standard deviations are plotted.

After 10 days of lens wearing (day 16), eyes treated with positive lenses (○) were hyperopic by $+4{\cdot}12 \pm 0{\cdot}54$ diopters, whereas eyes treated with negative lenses (●) were myopic by $-0{\cdot}64 \pm 0{\cdot}94$ diopters (Fig. 3*A*). The difference in refractions is significant (d.f. = 12, $t = 11{\cdot}62$, $P < 0{\cdot}001$, t test). Eyes treated with positive lenses grew slower in axial length (64 ± 11 μm day^{-1}) and were about 0·5 mm shorter after 10 days of lens wearing than eyes treated with negative lenses (growth rate:

113 ± 19 μm day^{-1}). The eyes differed significantly in axial lengths after only 3 days of lens treatment ($-0{\cdot}28 \pm 0{\cdot}21$ mm, d.f. = 12, $t = 3{\cdot}51$, $P < 0{\cdot}01$, t test). At day 16, the average axial length of the hyperopic eyes was $8{\cdot}40 \pm 0{\cdot}15$ mm, compared to the myopic eye which was $8{\cdot}88 \pm 0{\cdot}13$ mm in axial length ($n = 7$, d.f. = 12, $t = 6{\cdot}4$, $P <$

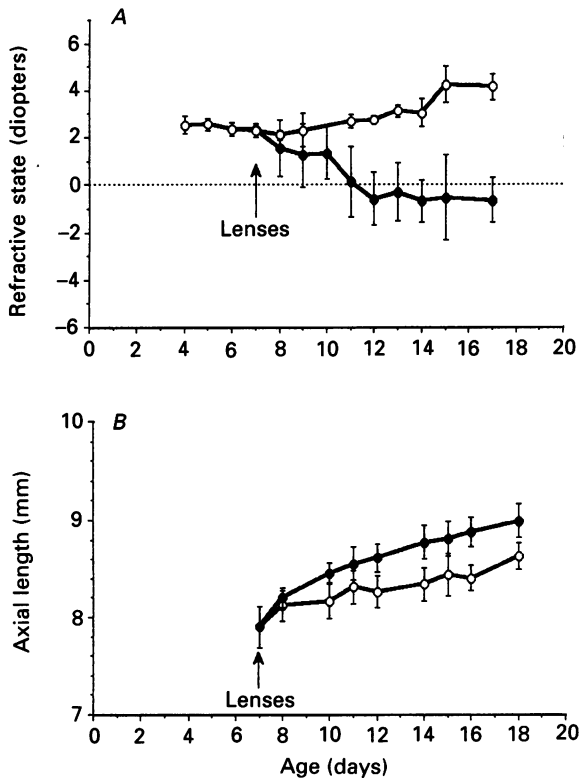

Fig. 3. Development of refractive state (A) and axial length (B) in a group of seven chickens raised in white light with different defocusing lenses in front of both eyes (+4 diopters, ○; −4 diopters, ●). Arrows denote the beginning of lens treatment. Error bars denote standard deviations from measurements of seven equally treated eyes. Note that the eyes change their refractive state and their axial growth rate in response to the lens treatment such that the imposed defocus is largely compensated.

0·001, t test). The other three parameters measured were not significantly different in the two differently treated eyes (anterior chamber depth, $+0{\cdot}03 \pm 0{\cdot}144$ mm, $t = 0{\cdot}55$, $P > 0{\cdot}5$, n.s.; corneal radius of curvature, $+0{\cdot}08 \pm 0{\cdot}12$ mm, $t = 1{\cdot}76$, $P < 0{\cdot}1$, n.s.; and thickness of the lens, $-0{\cdot}04 \pm 0{\cdot}08$ mm, $t = 1{\cdot}39$, $P < 0{\cdot}3$, n.s. (t test; $n = 7$, d.f. = 12 in all cases)). It can be concluded that refractive errors were axial in nature.

Experiments with lenses in red and blue light

To test whether emmetropizing mechanisms could sense the imposed defocus in deep red and near-ultraviolet illumination, we repeated the lens experiment for both light conditions. In both cases, seven chicks wearing lenses were used. Results are

shown in Figs 4 and 5. In deep red light, the results were not different (Fig. 4) from the ones obtained in white light (Fig. 3). Refractive state and axial length were significantly changed after only 2 days of lens wearing (difference in axial lengths $-0{\cdot}22 \pm 0{\cdot}21$ mm, $n = 7$, d.f. = 12, $t = 2{\cdot}71$, $P < 0{\cdot}02$, t test). Eyes treated with

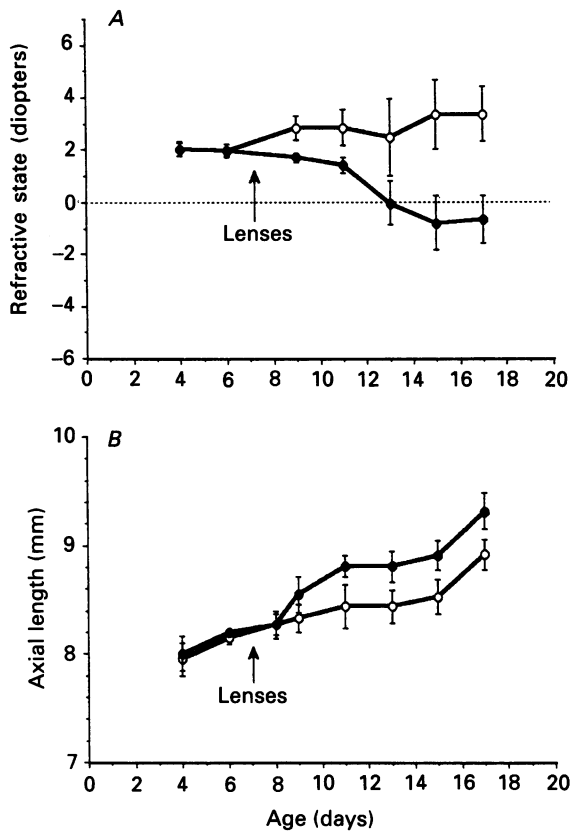

Fig. 4. A similar lens experiment to that shown in Fig. 3, but performed in deep red light. Number of chicks and plot as in Fig. 3. Note that the results are similar to the experiment performed in white light, indicating that emmetropization is functional with the long wavelength cones. ○, positive lenses; ●, negative lenses.

negative lenses became weakly myopic ($-0{\cdot}66 \pm 0{\cdot}91$ diopters) compared to eyes with positive lenses which became hyperopic ($+3{\cdot}36 \pm 1{\cdot}05$ diopters; Fig. 4*A*). Axial lengths also differed significantly by the end of the experiment (Fig. 4*B*): $8{\cdot}92 \pm 0{\cdot}14$ mm (hyperopic eyes), *versus* $9{\cdot}32 \pm 0{\cdot}16$ mm (myopic eyes), $t = 4{\cdot}97$, $P < 0{\cdot}001$; t test. There were no differences detected in the thickness of the lens, anterior chamber depth and corneal radius of curvature in the two oppositely treated eyes: anterior chamber depth, $+0{\cdot}1 \pm 0{\cdot}13$ mm, $t = 1{\cdot}96$, $P < 0{\cdot}1$, n.s.; corneal radius of curvature, $+0{\cdot}04 \pm 0{\cdot}13$ mm, $t = 0{\cdot}83$, $P < 0{\cdot}5$, n.s.; and thickness of the lens, $-0{\cdot}03 \pm 0{\cdot}20$ mm, $t = 0{\cdot}91$, $P < 0{\cdot}3$, n.s. (t test; $n = 7$, d.f. = 12).

Strikingly, the results were quite different for the group of chicks raised in near-ultraviolet light. It should be emphasized that the relative brightness of the near-

ultraviolet light for the chickens was similar to the red light. Results are shown in Fig. 5. The eyes remained slightly hyperopic throughout the experiment for both positive (○) and negative (●) lenses (Fig. 5*A*). They did not differ from eyes of animals raised in ultraviolet light without lenses (Fig. 2). Also axial length was not

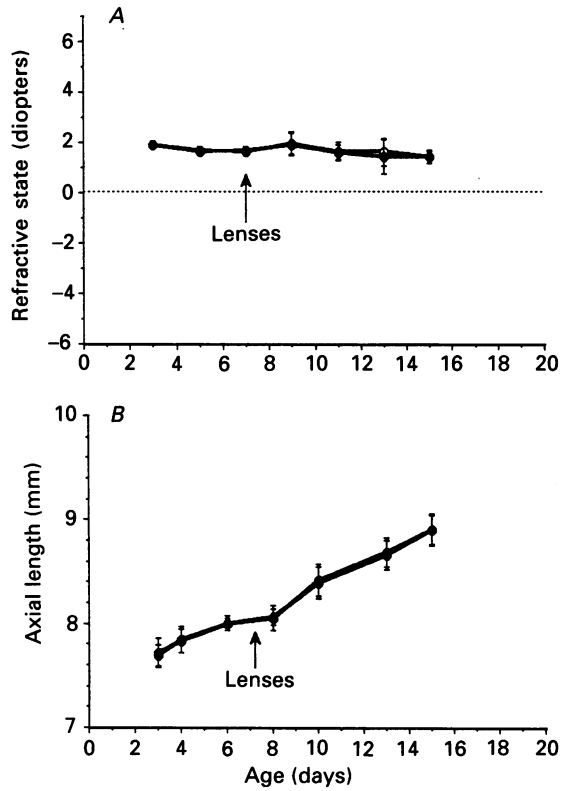

Fig. 5. Lens experiment performed in near-ultraviolet light of same relative brightness as the deep red light used for Fig. 4. Number of chicks and plot as in Fig. 3. Note that the lenses had no effect in the ultraviolet light, indicating that the short wavelength system cannot guide emmetropization. ○, positive lenses; ●, negative lenses.

different for lenses of different sign (Fig. 5*B*). The eyes grew linearly with no influence of the defocus provided by the lenses. The growth rate of the eyes (positive lenses, 101 ± 14 μm day^{-1}; negative lenses, 100 ± 16 μm day^{-1}) was not significantly different from untreated control animals (100 μm day^{-1}) as reported by Pickett-Seltner, Sivak & Pasternak (1988). None of the analysed parameters was significantly correlated with the powers of the lenses: refractions, $-0{\cdot}22 \pm 0{\cdot}89$ diopters, $t = 0{\cdot}65$, $P > 0{\cdot}5$, n.s.; axial length, $-0{\cdot}01 \pm 0{\cdot}18$ mm, $t = 0{\cdot}14$, $P > 0{\cdot}5$, n.s.; anterior chamber depth, $-0{\cdot}04 \pm 0{\cdot}12$ mm, $t = 0{\cdot}91$, $P > 0{\cdot}5$, n.s.; corneal radius of curvature, $-0{\cdot}01 \pm 0{\cdot}09$ mm, $t = 0{\cdot}29$, $P > 0{\cdot}5$, n.s.; and thickness of the ocular lens, $+0{\cdot}02 \pm 0{\cdot}09$ mm, $t = 0{\cdot}56$, $P > 0{\cdot}5$, n.s. (t test; for all parameters $n = 7$, d.f. = 12).

The results show that the ultraviolet-sensitive receptor system has either no input

to the feedback loops controlling refractive development, or its visual acuity is not sufficient to detect the defocus from the lenses, or both.

DISCUSSION

We have found that, despite about 3 diopters of longitudinal chromatic aberration, the chicken eye can adjust its refractive state during development for the mid-wavelength range. Such a performance could be expected if either only receptors from the mid-wavelength range feed into the feedback loops guiding emmetropization, or if all receptor systems have input but the ones with best acuity finally determine refractive state. Mandelman & Sivak (1983) provide data on the magnitude of longitudinal chromatic aberration: with 590 nm as a reference point of zero refraction, in humans chromatic defocus amounts to 2·08 diopters of myopia at 440 nm but only 0·65 diopters of hyperopia at 680 nm. In chicks, the function is slightly flatter with an (extrapolated) defocus of 1·3 diopters and 0·34 diopters, respectively. Because the chicken is sensitive down to 360 nm, by extrapolation of the data presented by Mandelman & Sivak (1983) a chromatic defocus of at least 3 diopters must be expected at this wavelength. With regard to the spectral sensitivity function of the chicken (Fig. 1), chromatic aberration amounts to about 1·4 diopters over the mid-wavelength range where the chicken is maximally sensitive. We have estimated the monochromatic depth of focus of the chicken eye by (1) the formulae given by Green, Pavers & Banks (1980), and (2) by the standard deviations from many measurements of refractive state in individual eyes. Both estimations give a depth of focus of about 1 diopter. Therefore, chromatic aberration in the spectral range covered by the four cone types (about 1·4 diopters) is similar to the monochromatic depth of focus of the eye. It is, therefore, not critical. The situation is different for the ultraviolet receptor, and, if this receptor system could guide emmetropization equally well it would have to take into account the apparent myopia of about 2 diopters from chromatic dispersion. We have therefore studied the visual acuity of the chicken ultraviolet receptor system.

Sampling intervals and spatial resolution of the ultraviolet system

To test whether the visual acuity of the chickens in the near-ultraviolet light was high enough to stimulate accommodation, chickens were videotaped with the infrared photoretinoscope while they were foraging. Single video frames were analysed off-line to measure refractive state. However, the chickens were found to accommodate normally during pecking and there was no obvious difference in pecking behaviour. There was a difference, however, when the lenses were in place. The chickens did not compensate for the lenses by accommodation as they did in previous experiments in white light (Schaeffel *et al.* 1988). It was concluded that the defocus imposed by the lenses was not detected, and that the spatial resolution of the ultraviolet system is poor.

The five cone types of the chickens are evenly distributed over the entire retina (Meyer & May, 1973), but are present in quite different numbers. Due to their coloured oil droplets, individual cone types can be easily distinguished in retinal flatmounts. The result of a 'nearest neighbour analysis' (Wässle & Riemann, 1978)

is shown in Fig. 6. From the average spacing, the 'theoretical anatomical resolving power, ARP' (Reymond, 1985), can be calculated for a single receptor system using the formula

$$\text{ARP (cycles deg}^{-1}) = \text{PND}/(\sqrt{3} \times d \times 57{\cdot}3),$$

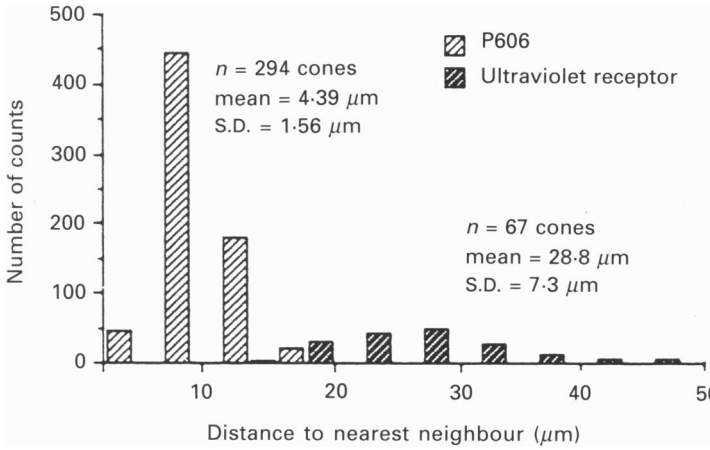

Fig. 6. Nearest neighbour analysis (Wässle & Riemann, 1978) for the long wavelength cones (P606) and the ultraviolet-sensitive cones. The absolute distances of the different cone types were determined in a retinal flatmount (sampled area 2·69 mm, from the central retina area). Cones can easily be identified in the light microscope by their coloured oil droplets. In order to distinguish between the clear oil droplets of the receptor P506 and the transparent oil droplet of the ultraviolet receptor, a short wavelength cut-off filter was placed into the light beam. Under these conditions, the oil droplets appeared blue and white, respectively. The L-cones (P606) are more narrowly distributed and outnumber the ultraviolet receptors by 4 times.

where PND is the posterior nodal distance of the eye (5·45 mm at 15 days; Schaeffel & Howland, 1988), d is the average centre-to-centre spacing of neighbouring cones (in mm), and 57·3 is the conversion factor from radians to degrees. Finally, from the anatomical resolving power (in cycles deg^{-1}) the depth of focus of the eye can be estimated by the formula $D = 21{\cdot}3/(\text{ARP}\ P)$, where D is depth of focus and P is the pupil diameter in millimetres (Braddick, Atkinson, French & Howland, 1979). The result of the estimation is that individual receptor classes in the mid- and long wavelength range have a depth of field of 1–1·5 diopters as opposed to the ultraviolet receptor system which has a depth of focus of 4–6 diopters, depending on pupil size.

Because the ultraviolet receptor system has a visual low acuity, we cannot decide whether the failure of the lens experiment (Fig. 5) is the result of the lenses being too weak to be detected or because the ultraviolet system does not contribute to the feedback loops controlling refractive state. Such a decision could be made by an experiment with more powerful lenses. However, in the chicken, feedback loops for regulation of refractive state are highly non-linear and are most sensitive to myopic defocus imposed by positive lenses (Schaeffel & Howland, 1991). Unfortunately, our positive lenses of higher power are quite thick and heavy and also induce a number of aberrations and distortions. They are therefore not tolerated by the chickens. On the other hand, more powerful negative lenses are not more efficient even in white

light because the response curve of the eye to change in its axial eye growth with increasing lens defocus levels off at −2 diopters. The question, therefore, cannot be answered by a second lens experiment with more powerful lenses of the type employed here. On the other hand, if the ultraviolet receptor system had no input to

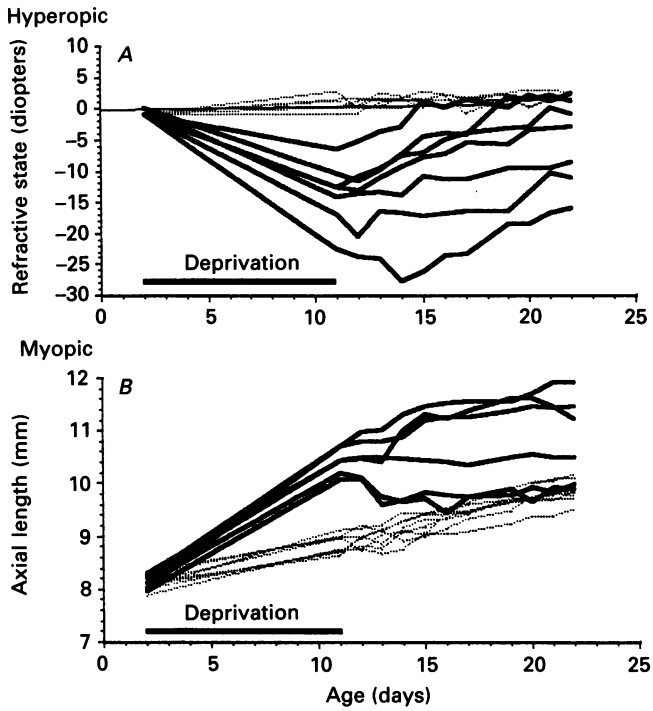

Fig. 7. Removing the high spatial frequencies from the retinal image in growing chicks and raising them in white light produces high amounts of myopia (*A*) which is axial in nature. Seven chickens were raised with monocular translucent occluders from day 2 to 11. Thick lines, occluded eyes; dotted lines, untreated contralateral control eyes. Standard deviations were omitted for clarity but were approximately +1 diopter for refractions (*A*) and 0·06 mm for axial length measurements (*B*). Upon removal of the occluders, most eyes quickly recover from myopia, mainly by an attenuation of axial eye growth. Myopia develops only if a lack of high spatial frequencies occurs in white light. In comparing Fig. 7 with Fig. 2, it can be seen that, in ultraviolet light, a normal refractive state is maintained for a similar period of time despite the lack of visual control of refractive development. The result indicates that an inappropriate stimulation of a high acuity system is necessary to produce an error signal for the eye to grow in excess.

the regulation of eye growth at all, the results for dark rearing and rearing in ultraviolet light should be similar. They are not, however. In the dark, chickens typically develop flat corneae, large eyes and hyperopic refractive errors (Gottlieb, Fugate-Wertzek & Wallman, 1987). Because the 'dark-rearing syndrome' was suppressed, some input of the ultraviolet receptor system on eye growth must be expected, although this input must not necessarily relate to emmetropization.

The observation, that refractive state was stable in the ultraviolet for more than a week despite the lack of visual information which could guide axial eye growth, is

intriguing with regard to deprivation myopia. Animals raised in white light but with occluders which remove the high spatial frequencies from the retinal image quickly develop high amounts of myopia (Fig. 7*A*) as a result of exaggerated axial eye growth (Fig. 7*B*). Due to the degraded retinal image, information on the current refractive state is not available, similar to the condition in ultraviolet light. Yet, deprivation myopia develops only in white light. It can be concluded that, if the high acuity systems are stimulated but with insufficient information regarding the focal plane, myopia develops. It does not develop if the high acuity systems are not stimulated at all. Therefore, deprivation myopia cannot just be considered to be the result of a lack of information on the current refractive state, but the lack of high spatial frequencies provided by the green and red cones must provide a real error signal to the high acuity system. This does not necessarily imply that both processes (occluder myopia and emmetropization with lenses) use the same subsequent pathways.

In conclusion the low visual acuity of the ultraviolet receptor system offers an elegant way for the eye to bypass the problem of multiple chromatic focal planes during emmetropization. However, it cannot be deduced whether the ultraviolet system has a higher threshold to provide an error signal for emmetropizing mechanisms or whether it does not feed into those mechanisms at all.

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