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Measurement of the photoreceptor pointing in the living chick eye

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ABSTRACT

The chick eye is used in the study of ocular growth and emmetropization; however optical aberrations in the lens and cornea limit the ability to visualize fine retinal structure in living eyes. These aberrations can be corrected using adaptive optics (AO) allowing for cellular level imaging *in vivo*. Here, this capability is extended to measure the angular tuning properties of individual photoreceptors.

The left eyes from two White Leghorn chicks (*Gallus gallus domesticus*) labeled chick A and chick B, were imaged using an AO flood illuminated fundus camera. By translating the entrance pupil position, the same retinal location was illuminated with light of varying angles allowing for the measurement of individual photoreceptor pointing. At 30° nasal from the pecten tip, the pointing direction for both chicks was towards the pupil center with a narrow distribution. These particular chicks were found to have a temporal (T) and inferior (I) bias in the alignment with peak positions of (0.81 T, 0.23 I) and (0.57 T, 0.18 I) mm from the pupil center for chicks A and B respectively. The rho, ρ, values for the major, ρ_L, and minor, ρ_s, axes were 0.14 and 0.17 mm^{-2} for chick A and 0.09 and 0.20 mm^{-2} for chick B. The small disarray in the alignment of the chick photoreceptors implies that the photoreceptors are aligned to optimize the light entering the eye through the central portion of the pupil aperture. The ability to measure pointing properties of individual photoreceptors will have application in the study of eye growth and various retinal disorders.

1. Introduction

Stiles and Crawford were the first to discover the directional sensitivity of cone photoreceptors, finding that light entering the eye at the pupil center appears brighter than the same intensity light incident near the pupil edge (Stiles & Crawford, 1933). This phenomenon is known as the Stiles–Crawford Effect of the First Kind (SCE-I) and is due to the waveguide properties of cone photoreceptors (Enoch, 1963; Westheimer, 2008).

In healthy human eyes, the photoreceptors are aligned towards the center of the pupil with a slight nasal bias of 0.5 mm with psychophysically determined ρ values of 0.05 mm^{-2} (Enoch & Tobey, 1981). Reflectometry based methods give higher ρ values ranging between 0.1 and 0.2 mm^{-2}. However, many retinal conditions exhibit a disruption in the SCE-I function; either (i) the position of the peak, (ii) the shape profile of the function or (iii) both can be altered depending on the underlying causes of the retinal change. For example, myopic eyes showed a systematic nasal shift in the peak position of the SCE-I function at the nasal retina with an increase in myopia. In other words, the alignment of cone photoreceptors was markedly skewed towards the optic nerve head at the nasal retina due to the tractional force associated with the axial elongation of the eye (Choi, Enoch, & Kono, 2004; Choi, Garner, & Enoch, 2003a). Such nasal bias at the nasal retina was found to be maintained even after the refractive surgery which further supports earlier findings that the skewing of the peak position of the SCE-I function has the origin at the retinal traction caused by the axial elongation of the eye (Choi, Garner, & Enoch, 2003b, 2003c). Marcos and Burns also studied the effect of LASIK on the SCE-I and found that in most cases, there were no significant changes to the SCE-I before and after the procedure with an exception of 2 eyes whose reason is unclear, and concluded that optical degradation is not a driving mechanism for cone orientation (Marcos & Burns, 2009).

Furthermore, SCE-I changes have been found in several retinal diseases such as central serous chorioretinopathy (Kanis & van Norren, 2008; Smith, Pokorny, & Diddie, 1978), retinitis pigmentosa (Birch & Sandberg, 1982; Birch, Sandberg, & Berson, 1982) and age related macular degeneration (Kanis et al., 2008; Smith, Pokorny, & Diddie, 1988).

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Other ocular conditions resulting in changes to the SCE-I function include macular edema (Lardenoye et al., 2000), fundus ectasia (Lakshminarayanan, Bailey, & Enoch, 1997), iris coloboma (Bailey, Lakshminarayanan, & Enoch, 1994), choroidal atrophy (Bedell, Enoch, & Fitzgerald, 1981), fundus flavimaculatus (Morse et al., 1981), and acute posterior multifocal placoid pigment epitheliopathy (Smith, Pokorny, Ernest, et al., 1978). Bresnick et al. showed changes in the SCE-I function in diabetic retinopathy (Bresnick, Smith, & Pokorny, 1981), however no changes were observed in diabetes mellitus (Zagers, Pot, & van Norren, 2005). Previous studies have supported the notion that the SCE-I has its origin at the retina, hence measuring the SCE-I function in patients is potentially a sensitive way of detecting photoreceptor cell disturbance in diseased retinas (Smith et al., 1988).

The SCE-I has also been studied in several different animal species, for example, the macaque (Matsumoto et al., 2012), turtle (Baylor & Fettiplace, 1975), blowfly (van Hateren, 1985), and the chick (Beresford, Crewther, & Crewther, 1999). Despite the species differences, the general consensus is that the photoreceptors are aligned towards a central area in the front of the eye such as the entrance pupil or the lens to optimize the light entering the eye. Various techniques have been used to measure the SCE-I function in animal eyes, ranging from histology to intracellular recording (in turtle and blowfly) to electroretinography (ERG) (in macaque and chick).

In human eyes, psychophysical techniques have been used most extensively, but require good concentration from subjects and are also time consuming procedures. More recently, objective techniques based on reflectometry have been used to measure the SCE-I function in humans (Gao et al., 2008; Gorrand & Delori, 1995; He, Marcos, & Burns, 1999; Van Blockland, 1986; van de Kraats & van Norren, 2008). Both techniques have been shown to provide similar estimates of the SCE-I peak position (i.e., the pointing direction), although the ρ value (i.e., the width of the distribution) from the reflectometric approach tends to be higher than that from psychophysical measurements. The discrepancy of the ρ values is thought to be caused by the difference in the nature of the light being analyzed, i.e., the reflectometry measurement relies on the light reflected off the retina whereas in the psychophysical technique, it measures the threshold intensity of light that falls on the retina as perceived by the eye.

Although individual cellular level analyses are possible from excised retina, they are prone to preparation artifacts; hence *in vivo* approaches would be the most accurate way of measuring the directional properties of individual cone photoreceptors. Recently, single retinal photoreceptors were imaged in the living chick eye using adaptive optics (AO) retinal imaging systems (Bueno et al., 2014; Headington et al., 2011; Kisilak et al., 2012). In this study, AO retinal imaging was used in conjunction with the cone pointing measurement technique of Roorda and Williams (2002) to determine the pointing of individual chick photoreceptors.

2. Methods

2.1. Animal preparation

Two, 48 day old White Leghorn chicks (*Gallus gallus domesticus*) were hatched and housed in the animal facility at the New England College of Optometry. Their housing units were temperature regulated and under a 12-h light/dark cycle (light from 8:30 am to 8:30 pm). Food and water were provided ad libitum. Table 1 outlines the physical and optical properties of the chicks. The animals were cared for in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, the New England College of Optometry Institutional Animal Care and Use Committee (IACUC) and the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Table 1
Physical and optical properties of the two chicks. Lateral resolution assumes a 3 mm pupil diameter and 680 nm imaging light.

	Chick A	Chick B
Age (days)	48	48
Weight (g)	570	600
Eye imaged	Left	Left
Pupil diameter (mm)	3.30	3.60
Corneal refractive power (diopters)	41.42	39.33
Anterior chamber thickness (mm)	1.55	1.74
Lens thickness (mm)	2.89	2.70
Vitreous chamber thickness (mm)	7.05	7.79
Retinal thickness (mm)	0.22	0.21
Axial length (mm)	11.71	12.44
Focal length (mm)	8.05	8.55
Lateral resolution (μm)	2.23	2.36

On the day of the AO retinal imaging, refractive error (Hartinger's refractometer) and ultrasound measurements (Panametrics Model 176599) were taken. Prior to anesthesia, birds were dilated using vecuronium bromide (1 mg/mL, Sigma–Aldrich, St. Louis, Missouri) resulting in dilated pupils of 3.3–3.6 mm in diameter. Anesthesia was administered through an intramuscular injection with a mixture containing 1 mg/kg ketamine and 0.2 mg/kg xylazine. Deep anesthesia was maintained during imaging with an extra half-dose of the anesthesia combination, if required. Both birds were mounted in a prone position onto a positioning assembly, securing the beak to ensure precise alignment of the pupil. As needed, non-preserved Celluvisc (Allergan, Inc., Irvine, CA, USA) artificial teardrops were applied to maintain adequate corneal hydration during the experiment. The described setup allowed for an imaging session of approximately 45 minutes in duration.

2.2. AO flood illuminated fundus camera

An AO flood illuminated fundus camera described by Headington et al. (2011) was utilized for all experiments with two important modifications, (i) the magnification of the last telescope was modified to allow for measurements over a 3 mm pupil diameter instead of the previous 2 mm and (ii) 680 nm light was used for imaging.

A fiber coupled SLD at 680 ± 20 nm was used as the imaging light source instead of the 550 ± 40 nm Hg–Xe arc lamp employed by Headington et al. While the use of 680 nm wavelength light results in images with slightly lower contrast than the 550 nm wavelength light, the higher power light source did allow for much faster image acquisition. This resulted in sets of images from the various entrance pupil positions with minimal eye motion and hence maximal image overlap. Bursts of 20 images were taken with an individual exposure duration of 10 ms and an interval of 40 ms giving a frame rate of ~20 Hz. The process was repeated if the images were not of sufficient quality. The iris was then translated to the next pupil position to image over the 25 entrance pupil positions. The power of the imaging light was 60 μW for the central pupil position measured at the corneal plane, however this varied by up to 50% at other pupil positions due to the collimation optics and the Gaussian nature of the imaging source. Intensities of individual images were therefore equalized based on automated readings from a computer interfaced power meter.

The refractive error of the eye was measured using a Hartinger's refractometer and then further refined by measuring the residual RMS error of the eye using the Shack–Hartmann wavefront sensor (WFS). The trial lens combination was adjusted to achieve a baseline RMS error of ~0.6 μm prior to the AO correction.