Vision Research 130 (2017) 57–66

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Evaluating outer segment length as a surrogate measure of peak foveal cone density

Melissa A. Wilk [a,1], Brandon M. Wilk [b], Christopher S. Langlo [a], Robert F. Cooper [c,2], Joseph Carroll [a,b,c,d,*]

[a] Department of Cell Biology, Neurobiology & Anatomy, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA
[b] Department of Ophthalmology, Medical College of Wisconsin, 925 N. 87th Street, Milwaukee, WI 53226, USA
[c] Department of Biomedical Engineering, Marquette University, 1250 W Wisconsin Avenue, Milwaukee, WI 53233, USA
[d] Department of Biophysics, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA

ARTICLE INFO

Article history:
Received 2 August 2016
Received in revised form 16 September 2016
Accepted 17 October 2016

Keywords:
Fovea
Cone photoreceptor
Adaptive optics
Outer segment
Cone density

ABSTRACT

Adaptive optics (AO) imaging tools enable direct visualization of the cone photoreceptor mosaic, which facilitates quantitative measurements such as cone density. However, in many individuals, low image quality or excessive eye movements precludes making such measures. As foveal cone specialization is associated with both increased density and outer segment (OS) elongation, we sought to examine whether OS length could be used as a surrogate measure of foveal cone density. The retinas of 43 subjects (23 normal and 20 albinism; aged 6–67 years) were examined. Peak foveal cone density was measured using confocal adaptive optics scanning light ophthalmoscopy (AOSLO), and OS length was measured using optical coherence tomography (OCT) and longitudinal reflectivity profile-based approach. Peak cone density ranged from 29,200 to 214,000 cones/mm^2 (111,700 ± 46,300 cones/mm^2); OS length ranged from 26.3 to 54.5 μm (40.5 ± 7.7 μm). Density was significantly correlated with OS length in albinism ($p < 0.0001$), but not normals ($p = 0.99$). A cubic model of density as a function of OS length was created based on histology and optimized to fit the albinism data. The model includes triangular cone packing, a cylindrical OS with a fixed volume of 136.6 μm^3, and a ratio of OS to inner segment width that increased linearly with increasing OS length ($R^2 = 0.72$). Normal subjects showed no apparent relationship between cone density and OS length. In the absence of adequate AOSLO imagery, OS length may be used to estimate cone density in patients with albinism. Whether this relationship exists in other patient populations with foveal hypoplasia (e.g., premature birth, aniridia, isolated foveal hypoplasia) remains to be seen.

1. Introduction

The human fovea underlies the majority of our visual function, including color vision and high spatial acuity. While the fovea occupies only about 0.02% of the total retinal area, some 40% of primary visual cortex is devoted to processing signals from it (Hendrickson, 2005). Several anatomical features distinguish the foveal region, namely an avascular zone (FAZ), the displacement of inner retinal neurons (forming the foveal pit), and a pronounced increase in cone density (with an absence of rod photoreceptors in the central fovea). Despite its importance for human vision, much remains to be discovered about how this structure develops, how it is disrupted during aging and disease, and how it interacts with central visual system structures to determine key features of visual function.

Several conditions are known to affect the development of the fovea. Individuals born prematurely have been shown to have smaller FAZs and foveal pits (Hammer et al., 2008; Wilk et al., 2014a; Yanni et al., 2012). In addition, patients with albinism or aniridia also have foveal hypoplasia (lack of a foveal pit) as well as reduced foveal cone specialization (Wilk et al., 2014a, 2014b). Other cases of isolated foveal hypoplasia in the absence of albinism/aniridia have also been described (Perez et al., 2014; Saffra, Agarwal, Chiang, Masini, & Bertolucci, 2012). While cone specialization hasn't been studied in some of these populations, data from albinism suggests that the lack of a foveal pit would result in reduced cone packing at the fovea (Wilk et al., 2014b). Insights into

* Corresponding author at: Department of Ophthalmology & Visual Sciences, Medical College of Wisconsin, 925 N. 87th Street, Milwaukee, WI 53226, USA.

E-mail addresses: mwilk@hudsonalpha.org (M.A. Wilk), wilkb777@gmail.com (B.M. Wilk), clanglo@mcw.edu (C.S. Langlo), rfcooper@sas.upenn.edu (R.F. Cooper), jcarroll@mcw.edu (J. Carroll).

[1] Present address: HudsonAlpha Institute for Biotechnology, 601 Genome Way NW, Huntsville, AL 35806, USA.

[2] Present address: Departments of Psychology & Ophthalmology, University of Pennsylvania, Philadelphia, PA 19104, USA.

http://dx.doi.org/10.1016/j.visres.2016.10.012
0042-6989/

the level of cone specialization in these subjects would not only give insight into foveal development, but also provide clues as to the cause of vision deficits in these individuals.

One of the key technological advances in our ability to study the human fovea has been non-invasive retinal imaging. For example, optical coherence tomography (OCT) can be used to examine foveal pit morphology (Chui, Zhong, Song, & Burns, 2012; Dubis, McAllister, & Carroll, 2009; Dubis et al., 2012; Hammer et al., 2008; Wagner-Schuman et al., 2011; Wilk et al., 2014b; Wilk et al., 2016) and the avascular zone (Braaf et al., 2013; Samara et al., 2015; Wilk et al., 2016). In addition, adaptive optics (AO) imaging enables direct visualization of individual rod and cone photoreceptors (Dubra et al., 2011; Li, Tiruveedhula, & Roorda, 2010; Putnam et al., 2005; Wilk et al., 2014b; Zhang et al., 2015). While there has been success in measuring peak cone density in normal populations (Putnam et al., 2005; Wilk et al., 2014b; Wilk et al., 2016; Zhang et al., 2015), the presence of nystagmus in a range of retinal diseases often precludes high-resolution imaging (Langlo et al., 2016; Wilk et al., 2014b). With the goal of relating foveal cone structure to visual system function (Rossi & Roorda, 2010; Williams & Coletta, 1987), these limitations represent an important barrier in vision research.

A review of foveal cone anatomy provides clues as to possible alternative strategies for estimating foveal cone density. As mentioned above, it is widely appreciated that the fovea contains the highest density of cone photoreceptors in the normal human retina, with estimates ranging from 80,000 to 300,000 (Curcio, Sloan, Kalina, & Hendrickson, 1990; Gao & Hollyfield, 1992; Putnam et al., 2005; Wilk et al., 2014b; Wilk et al., 2016; Yuodelis & Hendrickson, 1986; Zhang et al., 2015). Moreover, it has been demonstrated by numerous investigators using ex vivo (Yuodelis & Hendrickson, 1986) and in vivo (Hammer et al., 2008; Liu, Kocaoglu, Turner, & Miller, 2015; McAllister et al., 2010; Wilk et al., 2014b) techniques that foveal cone outer segments (OS) are elongated relative to peripheral cones. It has been suggested that the elongation and increased packing of cones are directly linked (Diaz-Araya & Provis, 1992; Hendrickson & Yuodelis, 1984; Provis, Dubis, Maddess, & Carroll, 2013). As the cones become tightly packed, the OS diameter decreases; since the OS appears to have constant volume (Hoang, Linsenmeier, Chung, & Curcio, 2002), the OS elongates to fit into the tight packing array. It is this concept that formed the basis for the present study. Here, we used AO scanning light ophthalmoscopy (AOSLO) and OCT to examine cone density and OS elongation in subjects with a range of cone densities. These data were then used to adapt a model for the relationship between peak density and OS length, which can be used to estimate foveal cone density from OS length in patients with albinism. Given the relative ease of measuring OS length compared to cone density, as well as the broader access to OCT compared to AO imaging devices, this could be a useful approach for vision scientists to characterize foveal cone specialization in difficult populations.

2. Methods

2.1. Subjects

This study followed the tenets of the Declaration of Helsinki and was approved by the Medical College of Wisconsin Institutional Review Board. Informed consent was obtained from all subjects (or adult guardian of minors) after explanation of the nature and possible consequences of the study. Twenty-three subjects with normal vision (7 female, 16 male; 8–67 years of age) and 20 subjects with albinism (9 male, 11 female; 6–40 years of age) were recruited for this study (Table 1). A subset of the subjects had previously participated in studies by Wilk et al. (2014b) and/or Cooper, Wilk, Tarima, and Carroll (2016). All normal subjects except JC_0878 have also been described by Wilk et al. (2016). Each subject had one eye dilated and accommodation suspended using one drop each of Phenylephrine Hydrochloride (2.5%) and Tropicamide (1%) prior to imaging. Axial length, used for estimating the absolute scale of the retinal images, was measured using an IOL Master (Carl Zeiss Meditec, Dublin, CA).

2.2. Measuring foveal cone density

The foveal cone mosaic was imaged using confocal reflectance AOSLO (Dubra et al., 2011). The AOSLO image sequences were registered and averaged as previously described to create images with high signal-to-noise ratios (Cooper et al., 2011; Dubra & Harvey, 2010). Peak cone density was estimated using a previously described method (Wilk et al., 2014b). Briefly, a region encompassing the peak density was cropped from the foveal images. Cones in the image were semi-automatically identified as previously described (Garrioch et al., 2012). The density at each pixel in the image was computed by counting the cones within variable window sizes. The densities at each pixel for all window sizes were averaged, and the pixel with the greatest average density was considered the location of peak density. The density at this location was then measured using a 37×37 μm sampling window and recorded as the peak foveal cone density.

2.3. Estimating foveal outer segment (OS) length

High-resolution SD-OCT (Bioptigen, Research Triangle Park, NC) was performed on all subjects. Horizontal line scan sets were acquired (1000 A-scans/B-scan; 100–200 repeated B-scans; nominal scan length of 6 or 7 mm) through the foveal center. When a normal pit was absent (e.g., albinism), imaging was centered at the location of the incipient fovea (based on inspection of additional volumetric scans obtained). Line scans were registered and averaged as previously described to reduce speckle noise in the image (Tanna et al., 2010). The lateral image dimension was corrected for axial length by dividing the nominal scan width by the assumed axial length of the device (24 mm) times the subject's actual axial length. Processed line scans were cross-referenced with volumetric scans (ranging from 400 to 750 A-scans/B-scan and 100–250 B-scans over 6×6 or 7×7 mm nominally) to confirm that the location of apparent maximum OS length was encompassed in the line scan. In the eight subjects for whom this was not the case (Table 1), a single B-scan from the volume scan was used for analysis. In subjects with albinism lacking true OS elongation ($n = 4$), the fovea was identified by other features such as outer nuclear layer thickening or doming of the retina as described by McAllister et al. (2010).

Custom Java (Oracle Corporation, Redwood Shores, CA) software was written for analysis of OCT reflectance through use of longitudinal reflectivity profiles, or LRPs (OCT Reflectivity Analytics [ORA], Fig. A.1). Prior to analysis, each subject's image was resampled so that all images were the same scale and dimensions in both directions, and all LRPs were created over a 5-pixel (26.3 μm) width from the linear image. The location estimated to be the greatest OS length was manually selected for each image by a single observer (MAW). Consecutive LRPs were generated every 25 μm over a 500-μm region centered on this selection. For each LRP, the user selected the peaks corresponding to the EZ and IZ bands (Fig. 1). The distance between the two peaks for each LRP was calculated. A Gaussian was fit to the difference between peaks over the 500-μm width to generate an interpolated contour of OS length for this region. The reported maximum OS length is the maximum of the