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Postnatal maturation of the fovea in *Macaca mulatta* using optical coherence tomography

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ABSTRACT

Changes in the foveal anatomy during infancy are an important component in early development of spatial vision. The present longitudinal study in rhesus monkeys was undertaken to characterize the postnatal maturation of the fovea. Starting at four weeks after birth, the retinas of the left eyes of sixteen infant monkeys were imaged using spectral domain optical coherence tomography (SD OCT). Retinal scans were repeated every 30 days during the first year of life and every 60 days thereafter. Volume scans through the fovea were registered, scaled using a three surface schematic eye, and analyzed to measure foveal pit parameters. The individual layers of the retina were manually segmented and thicknesses were measured over a transverse distance of 1250 microns from the center of the foveal pit. Based on infrared scanning laser ophthalmoscope (IR SLO) images acquired with the SD OCT system, there were significant changes in the extent of the retina scanned as the eyes matured. Using a three-surface schematic eye, the length of each scan could be computed and was validated using image registration ($R^2 = 0.88$, slope = 1.003, $p < 0.05$). Over the first 18 months of life, the mean retinal thickness at the pit center had increased by 21.4% with a corresponding 20.3% decrease in pit depth. The major changes occurred within the first 120 days, but did not stabilize until a year after birth. In *Macaca mulatta* infants, the primary anatomical maturation of the fovea occurs within the first few months of life, as determined by longitudinal data from SD OCT measurements. The timelines for maturation of the fovea correspond well with the normal development of the lateral geniculate nucleus, cortical neurophysiology, and spatial resolution in monkeys.

1. Introduction

In primates, the visual system is relatively immature at birth, with high contrast visual acuity often measuring less than 5 cycles per degree (cpd) in both humans and monkeys, compared to acuities of 30–40 cpd in adults (Boothe et al., 1985; Kiorpes and Movshon, 1998; Movshon et al., 2005; Norcia et al., 1990; Ordy et al., 1965; Teller, 1997). The visual system improves rapidly over the first few months of life in both species, at about the same species- adjusted relative rate (Boothe et al., 1985, 1988), until adult-like levels are reached at about five years of age for children (Ellemberg et al., 1999; Mayer and Dobson, 1982; Teller, 1997) or 40 weeks of age for monkeys (Kiorpes, 1992; Kiorpes and Kiper, 1996). The improvement of visual performance early in life has been attributed to changes in the eye's optics and retinal anatomy, along with neurologic maturation of the of afferent visual pathway (lateral geniculate nucleus and visual cortex, reviewed in Simona and National Research Council Committee on Vision (Simons and National Research Council (U.S.). Committee on Vision., 1993) and Wener and Chalupa (Werner and Chalupa, 2004)). The majority of investigations of early changes in visual optics and development of the fovea pit have been in old world monkeys, because of their similarity to humans.

Optical characteristics of the monkey eye have been measured and modeled by schematic eyes (Jacobs and Blakemore, 1988), double-pass ophthalmoscopy (Williams and Booth, 1981) and wavefront technology (Ramamirtham et al., 2006). These studies have shown that in primate eyes, the optical properties, including clarity and higher order aberrations, continue to improve up to 13–21 weeks of age. However, at birth the optics are relatively good and not considered a significant limit on visual resolution (Ramamirtham et al., 2006, 2007; Williams and Booth, 1981). In

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contrast, changes in ocular biometry, including axial length, corneal curvature and crystalline lens parameters, all of which have a significant influence on retinal magnification, and hence spatial resolution, develop over longer periods of early life. Specifically, a combination of retinal magnification and cone spacing in the fovea pit predict a five-fold increase in spatial resolution during the first two years of life (Hendrickson and Kupfer, 1976; Jacobs and Blakemore, 1988; Packer et al., 1990).

Resolution limits, based on photoreceptor characteristics and density in the fovea pit also have been investigated in the development of visual acuity. Although the future fovea of the monkey eye can be identified as early as fifty days post-conception (Hendrickson and Kupfer, 1976), it is relatively immature at birth, with only a single or bilayer of cuboidal or columnar cone cells in the neonatal macaque foveal pit (Hendrickson, 1992; Provis et al., 1998; Springer and Hendrickson, 2004a). The corresponding peak cone density at birth in monkeys is 31–41% (Hendrickson, 1992), whereas in humans it is only 17% (Yuodelis and Hendrickson, 1986) that of the adult fovea (Hendrickson, 1993). Within the first year after birth, peak cone density for monkeys increases from 43,000 cones/mm^2 to 210,000 cones/mm^2 (Packer et al., 1990). In humans, cone density has been measured at 36,294 cones/mm^2 at 5 days postnatal, and increases to 108,439 cones/mm^2 by 45 months of age, but is still not adult like (208,203 cones/mm^2) (Yuodelis and Hendrickson, 1986). The increase in cone density does not represent active mitosis (La Vail et al., 1991; Yuodelis and Hendrickson, 1986) but is a direct result of cone migration as is evident by a decrease in the rod free zone (Hendrickson and Kupfer, 1976; Packer et al., 1990). In addition the cone photoreceptors within the fovea become narrower and longer (inner segment diameter of 2 μm and 30–35 μm in length, while outer segments lengthen to 50–65 μm) (Hendrickson and Provis, 2006; Packer et al., 1990). These changes in the photoreceptors are thought to improve both the waveguide characteristics and efficiency of photon capture (Banks and Bennett, 1988; Wilson, 1988).

The retina can be assessed using non-invasive *in vivo* optical coherence tomography (OCT) imaging (Huang et al., 1991). The feasibility of using this technology for imaging the infant eye has been demonstrated by several investigators (Dubis et al., 2012; Lee et al., 2015; Maldonado et al., 2011; Rosen et al., 2015; Vajzovic et al., 2012; Vinekar et al., 2015), and several of these studies have investigated the maturation of the foveal region. However, the current understanding is primarily based on cross sectional data from histological (Hendrickson and Kupfer, 1976; Kiorpes et al., 2003; Packer et al., 1990; Springer and Hendrickson, 2005) and imaging studies that have only short follow-up times. Hence, to establish accurate fovea maturational rates and trends, data from multiple time points for a larger number of subjects are needed. Although OCT imaging in comparison to histology is limited by resolution (i.e. cannot resolve individual cells using conventional imaging), it allows for longitudinal follow up, with accurate change analysis of morphology and retinal layers. As with most optical retinal imaging methodologies, the accuracy of OCT transverse measurements is dependent on the optics of the eye. Although schematic eyes have been used to compute relative retinal magnification (Bengtsson and Krakau, 1992; Bennett et al., 1994; Garway-Heath et al., 1998; Holden and Fitzke, 1988; Jacobs and Blakemore, 1988; Littmann, 1982; Maldonado et al., 2010; Patel et al., 2011; Qiao-Grider et al., 2007), they have not been validated in a growing non-human primate infant eye. The purpose of this study was to document the retinal layer changes in the maturing infant primate eye using OCT technology after compensation for changes in ocular magnification. Some of the findings were reported previously in abstract form (N Patel et al. IOVS 2009; 50: ARVO E-Abstract 6207).

2. Methods

2.1. Subjects

The subjects for longitudinal follow up were sixteen healthy full-term infant rhesus monkeys (*Macaca mulatta*). Housing and rearing for the infants have been previously described (Hung et al., 1995; Smith and Hung, 1999; Smith et al., 2013). For histological correspondence to OCT, 2 animals; an infant 10 days of age that was born full-term and a 6yr old young adult with no prior experimental intervention were used. All experimental and animal care procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Houston. The use of animals for these experiments confirmed to National Institutes of Health guidelines for the care and use of laboratory animals.

Animals used for the longitudinal follow up were also subjects for studies of refractive error development (Huang et al., 2009; Smith et al., 2009a, 2009b, 2013) and, therefore, only the left, control eyes were used for analyses, i.e., eyes that had no optical manipulation that could interfere with normal post-natal development of the eye. High myopia secondary to form deprivation has been associated with retinal thinning (Abbott et al., 2011), however, none of the subjects for this study developed excess myopia. Of the 16 animals in this study, 12 had selective hemi-field intervention in the right eye that did not exceed 3D, and 4 were normal controls with no optical intervention (details of refractive outcomes have been presented previously (Smith et al., 2009a, 2013, 2010)). Overall, there were no nasal temporal OCT structural asymmetries noted in any of the findings on the control eye data (see results section). The initial OCT macula scans were acquired around thirty days of age (36 ± 6 days) and, subsequently, every thirty days thereafter for the first year of life. After one year of age the eyes were scanned at sixty-day intervals until the animals were at least 1.5 years of age (627 ± 27 days).

2.2. Animal preparation

Animals less than a year of age were anesthetized with an intramuscular injection of ketamine (15–20 mg/kg) and acepromazine maleate (0.15–0.2 mg/kg), while animals over a year of age were administered ketamine (20–25 mg/kg) and xylazine (0.4–0.6 mg/kg). Body temperature was maintained between 37 and 38 degrees Celsius with a thermostatically controlled electric blanket (TC1000 temperature controller, CWE, Ardmore, PA). Heart rate and blood oxygen were monitored with a pulse oximeter (model 9847 V; Nonin Medical Inc, Plymouth, MN). Prior to imaging the retina, the pupils were dilated with topical tropicamide (1%) and phenylephrine (2.5%), and a plano-powered rigid gas permeable contact lens with similar back surface curvature as the cornea was placed on the eye to maintain optical clarity. Head stabilization was achieved using mouth and occipital bars attached to a rotational mount, enabling appropriate eye alignment for scanning.

2.3. Optical coherence tomography

All scans were acquired using the Spectralis OCT + HRA (Heidelberg Engineering, Heidelberg, Germany) system. At high resolution setting, the infra-red scanning laser ophthalmoscope (IR SLO) captures frames at 5 Hz, whereas OCT A-scans are acquired at 40,000 Hz, with 1536 A-scans per 30°. Built in active eye tracking with this system minimizes eye movement artifact and allows for successive scan averaging, increasing the signal-to-noise ratio. The active eye tracking also makes this system ideal for follow up scans scanning identical regions overtime. However, in preliminary studies, due to eye growth and changes in ocular magnification, the