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Neonatal Aphakia is Associated with Altered Levels of Dopamine Metabolites in the Non-Human Primate Retina

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Abstract

Neonatal aphakia is associated with retardation of the axial elongation of the neonatal eye. In contrast, form deprivation increases axial elongation, an effect that has been associated with decreased retinal dopamine metabolism. The present investigation was conducted to test the hypothesis that neonatal aphakia induces an effect on the levels of retinal dopamine opposite to form deprivation. Lensectomy and vitrectomy were performed on the right eyes of rhesus monkeys at approximately 1 week of age; their left eyes were unmanipulated. Axial length was measured by A-scan ultrasonography. Prior to surgery, mean axial length of the right and left eyes was identical. Following lens removal, both eyes continued to elongate, however the aphakic eyes elongated at a slower rate resulting in a significant shorter axial length compared to that of the unmanipulated eye. Removal of the crystalline lens had no effect on steady-state dopamine levels in either central or peripheral retina. However, levels of the dopamine metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid were significantly elevated in central retina, but not in the peripheral retina of aphakic eyes. Our results support the hypothesis that dopamine is a component of the retinal signaling pathways that are involved in the regulation of eye growth and emmetropization.

Visual experience influences postnatal ocular growth and the development of emmetropia. In animal models, deprivation of form vision by lid fusion or occlusion results in an increase of axial elongation of the eye and induces a myopic shift in refraction (Raviola and Wiesel, 1985; Wallman and Adams, 1987). It is generally thought that form deprivation myopia is induced locally within the eye and occurs asymmetrically in eyes in response to partial visual field occlusion. While the retinal mechanisms associated with form deprivation-induced myopia (FDM) are poorly understood, dopamine has been identified as a candidate retinal modulator of postnatal ocular growth. In both chicks and monkeys, retinal dopamine metabolism is decreased under conditions that induce FDM (Iuvone et al., 1989) and the degree of axial elongation induced by deprivation is attenuated by apomorphine, a dopamine receptor agonist (Iuvone et al., 1991; Rohrer et al., 1993; Stone et al., 1989).

In contrast to form deprivation, surgical removal of the lens of neonatal monkeys retards the axial elongation of the eye (Lambert et al., 1996) and also alters scleral gene expression

(Tarnuzzer et al., 2005). The current study was undertaken to test the hypothesis that dopamine metabolism is altered by aphakia, consistent with its proposed role as a retinal regulator of postnatal ocular growth.

Rhesus monkeys (*Macaca mulatta*) were born at the Yerkes National Primate Research Center breeding facility. On the day of birth, they had complete ophthalmologic examinations and A-scan ultrasound axial eye length measurements. Animals were housed in individual isolates under a daily cycle of 10 hours light and 14 hours dark. At approximately 1 week of age, the lens of the right eye was removed by an established protocol (Lambert et al., 1996). Postoperative care included frequent inspections, twice daily antibiotic ointment, 1% atropine sulfate solution topically once a day for five days, and flunixin meglumine (Banamine; 1 mg/kg) intramuscularly four times daily for five days. Atropine was applied topically to prevent pupillary membranes from forming during the immediate postoperative period. All procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Emory University Institutional Animal Care and Use Committee.

Axial length measurements: Axial eye length measurements of anesthetized, cyclopleged monkeys were made by two ophthalmologists (SRL and AF) as described in detail by Tarnuzzer et al (Tarnuzzer et al., 2005) once a week.

When the axial length measurement of the unoperated phakic eye was 0.5 mm longer than the operated aphakic eye, monkeys were sacrificed mid-afternoon and the eyes were removed, placed in ice cold phosphate buffered saline, and sectioned slightly posterior to the pars plana. The vitreous was removed and the eye cup was flattened with radial cuts. A 0.7 cm trephine centered over the optic disc was used to separate the central retina from peripheral retina, which were gently separated from the retinal pigment epithelium and frozen on dry ice. The time from enucleation to freezing did not exceed 3 min. The samples were stored at -80°C until assayed for dopamine.

For analyses of dopamine, DOPAC, and HVA, frozen retinal samples were homogenized by sonication in ice cold 0.2 N HClO_4 containing 10 μM ascorbic acid and 20 ng/ml of 3,4-dihydroxybenzoic acid as internal standard. Homogenates were centrifuged at $15,000\times g$ for 15 minutes at 4°C . Protein in the pellet was assayed by the method of Lowry et al (Lowry et al., 1951). Dopamine, DOPAC, and HVA in the supernatant fraction were assayed by high performance liquid chromatography with electrochemical detection, as described previously (Iuvone et al., 1989).

Prior to lensectomy, the axial length measurements of the left and right eyes were not different (OD: 13.5 ± 0.1 mm; OS: 13.6 ± 0.1 mm) and similar to age-matched normal values (Bradley et al., 1999). However, within approximately six weeks after surgical removal of the lens, the aphakic eyes were significantly shorter than the phakic eyes (OD: 14.3 ± 0.3 mm; OS: 15.1 ± 0.2 mm; paired t-test, $p < 0.001$).

The steady state dopamine levels did not differ in either the central or the peripheral retina between the aphakic and the phakic eyes (Figure). However, the levels of the dopamine metabolites, DOPAC and HVA were 56% and 43% higher, respectively, in the central retina

of aphakic eyes compared to the central retina of the phakic eyes (DOPAC, $p=0.032$; HVA, $p<0.001$). In contrast, no differences in peripheral retina DOPAC and HVA levels were observed between aphakic and phakic eyes.

Previous studies have reported decreases in retinal dopamine metabolism in association with experimentally-induced excessive ocular elongation (Dong et al., 2011; McBrien et al., 2001; Stone et al., 1989). The current study found increased levels of dopamine metabolites in association with an experimental manipulation, surgical removal of the lens, which results in a decrease of the postnatal axial eye growth. Moreover, the current study showed that these changes are restricted to the central retina.

It has been shown that lens extraction during infancy results in a retardation of axial ocular growth (Boothe et al., 1996; Kugelberg et al., 1996; Lambert et al., 1995), and the effect is age-dependent. Lambert (Lambert, 1998) reported retardation in axial eye growth in newborn monkeys after lens extraction, but little effect if surgery was delayed to 7.5 months or 12 months of age. Other experimental manipulations that have been shown to retard axial eye growth include wearing a positive lens (myopic defocus) and increased ambient illumination. One of the mechanisms that has been proposed to account for reduced axial eye growth associated with both myopic defocus (Guo et al., 1995) and increased ambient illumination (Cohen et al., 2012; Rose et al., 2008) is elevated levels of dopamine and its metabolites in the retina. Our study, suggests that dopamine metabolites may be associated with the reduced axial eye growth associated with lens extraction during infancy. It is possible that a factor secreted by the crystalline lens inhibits dopamine activity. Therefore, removing the crystalline lens may increase dopamine activity leading to a rise in dopamine metabolites in the central retina. It is also possible that removing the lens may increase retinal dopamine activity by causing more light to reach the retina because the pupils are less reactive.

It has been proposed that dopamine functions as a “blur detector” in retina-mediated postnatal ocular growth (Feldkaemper and Schaeffel, 2013) and apomorphine, a dopamine receptor agonist, retards ocular elongation in visually-deprived chick and monkey eyes (Rohrer et al., 1993; Stone et al., 1989). Thus, the increase in retinal dopamine metabolism associated with aphakia may be causally related to a reduction in the axial eye growth of aphakic eyes, or may be a response to decreased axial elongation.

In our study, we found that dopamine metabolites, but not steady-state levels of dopamine were elevated in the central retina of aphakic neonatal monkeys. Steady-state levels of dopamine are tightly regulated, such that increased activity of dopamine neurons and release of dopamine is compensated by increased dopamine synthesis, maintaining relatively stable levels of dopamine (Brodie et al., 1966; Iuvone et al., 1978; Roth et al., 1974). Changes in dopamine metabolite levels in response to changes in neuronal activity are generally greater than changes in steady-state dopamine levels. For example, light-evoked increases of dopamine synthesis and turnover and of dopamine metabolite levels are much greater than any change in dopamine levels (Iuvone et al., 1978; Nir et al., 2000). In *rd1*, *rd5* and *nob* mice, DOPAC levels are dramatically reduced compared to that in wild-type controls, with no difference in steady-state dopamine levels (Nir and Iuvone, 1994; Pardue et al., 2008;

Park et al., 2013). Furthermore, form-deprivation in chicks results much greater decreases of DOPAC levels than of dopamine levels (Stone et al., 1989). Thus, our results showing higher levels of dopamine metabolites in central retina of aphakic eyes compared to retinas of phakic eyes without any significant difference in dopamine levels is consistent with previous studies.

In the Rhesus monkey, dopamine-containing amacrine cells are most numerous 3mm from the center of the fovea (Mariani et al., 1984), within the region we collected as central retina, with lower cell density in the peripheral retina. We did not find elevated levels of dopamine or its metabolites in the peripheral retina of the aphakic eyes. This may be because of the lower density of dopamine-containing amacrine cells in the peripheral retina or it may reflect a greater effect of lens extraction on dopamine production in the amacrine cells in the central than the peripheral retina.

Our data and the preceding studies are consistent with the hypotheses that dopamine is a modulator of postnatal eye growth and refractive development. Decreased dopamine neuronal activity is associated with excessive axial elongation of the eye, and increased dopamine neuronal activity is associated with decreased axial eye growth, at least in neonatal aphakic monkeys. However, further studies are necessary to prove that dopamine is the causative agent modulating these changes.

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Figure. Dopamine and Dopamine Metabolite Levels in Central and Peripheral Retina

Neonatal monkeys were subjected to unilateral lensectomy as described in Methods. Retinas were dissected when the difference in axial length between the unoperated and aphakic eyes was 0.5 mm. Compared to contralateral, unoperated eyes, the dopamine metabolites, DOPAC (* $p < 0.05$) and HVA (** $p < 0.001$), levels were significantly higher in central retina of aphakic eyes. No differences in dopamine metabolite levels were observed between

unmanipulated and aphakic eyes in the peripheral retina. No differences in dopamine levels were observed in either central or peripheral retina (n=5).

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