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# Opposing effects of atropine and timolol on the color and luminance emmetropization mechanisms in chicks

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ABSTRACT

This study analyzed the luminance and color emmetropization response in chicks treated with the non-selective parasympathetic antagonist atropine and the sympathetic β-receptor blocker timolol.

Chicks were binocularly exposed (8 h/day) for 4 days to one of three illumination conditions: 2 Hz sinusoidal luminance flicker, 2 Hz sinusoidal blue/yellow color flicker, or steady light (mean 680 lux). Atropine experiments involved monocular daily injections of either 20 μl of atropine (18 nmol) or 20 μl of phosphate-buffered saline. Timolol experiments involved monocular daily applications of 2 drops of 0.5% timolol or 2 drops of distilled $H_2O$. Changes in the experimental eye were compared with those in the fellow eye after correction for the effects of saline/water treatments.

Atropine caused a reduction in axial length with both luminance flicker (−0.078 ± 0.021 mm) and color flicker (−0.054 ± 0.017 mm), and a reduction in vitreous chamber depth with luminance flicker (−0.095 ± 0.023 mm), evoking a hyperopic shift in refraction (3.40 ± 1.77 D). Timolol produced an increase in axial length with luminance flicker (0.045 ± 0.030 mm) and a myopic shift in refraction (−4.07 ± 0.92 D), while color flicker caused a significant decrease in axial length (−0.046 ± 0.017 mm) that was associated with choroidal thinning (−0.046 ± 0.015 mm).

The opposing effects on growth and refraction seen with atropine and timolol suggest a balancing mechanism between the parasympathetic and β-receptor mediated sympathetic system through stimulation of the retina with luminance and color contrast.



## 1. Introduction

The increasing occurrence of myopia in the population presents an important public health issue because of an association with elevated risk of ocular diseases including cataract, glaucoma, retinal detachment, and blindness (Saw, Gazzard, Shih-Yen, & Chua, 2005). In humans, the environmental effect of time spent outdoors has been implicated in a reduction in myopia development (Dirani et al., 2009; Guggenheim et al., 2012; Jones et al., 2007; Jones-Jordan et al., 2012; Onal et al., 2007; Parssinen & Lyyra, 1993; Rose, Morgan, Ip, et al., 2008; Rose, Morgan, Smith, et al., 2008; Wu, Tsai, Hu, & Yang, 2010), and recent work has helped to clarify the protective effects of factors such as high light levels (chicks: Ashby, Ohlendorf, and Schaeffel (2009), Backhouse, Collins, and Phillips (2013), Cohen, Belkin, Yehezkel, Avni, and Polat (2008) and Cohen, Belkin, Yehezkel, Solomon, and Polat (2011); monkeys: Smith, Hung, and Huang (2012)) and spatial and temporal changes in the retinal image (Rucker, 2013; Rucker, Britton, Spatcher, & Hanowsky, 2015; Rucker & Wallman, 2008, 2009; Rucker & Wallman, 2012) that may be involved. In the meantime, promising pharmacological interventions (e.g., atropine) can slow the development of myopia progression (Chia et al., 2014, 2015, 2012; Bedrossian, 1971; Lee et al., 2006; Li et al., 2014; Morgan, Ohno-Matsui, & Saw, 2012; Walline, 2016; Wu, Yang, & Fang, 2011), although the effects of these treatments under different environmental conditions have not been studied.

### 1.1. Color and luminance contrast affect emmetropization

As a result of dispersion, short-wavelength light has a shorter focal length than long-wavelength light, producing an effect called longitudinal chromatic aberration. The differences in focus of the different wavelengths produce changes in color of the retinal image with defocus (Rucker & Wallman, 2012), which in turn is reflected in changes in the stimulation of the retinal cones and the retinotectal color and luminance pathways (review: Rucker (2013)). A theoretical analysis of the change in the retinal image with defocus has indicated that with myopic defocus, the retina would experience changes in luminance contrast, whereas with

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hyperopic defocus the retina would also experience changes in color contrast (Rucker & Wallman, 2009). In the laboratory, flickering light of fixed frequency and waveform can be used to simulate the changes in luminance and color contrast of the retinal image that occur with changes in focus and changes in fixation in the natural environment. Rucker and Wallman (2012) tested their hypothesis by exposing chicks to 2 Hz, high contrast, sinusoidal changes in either luminance or color contrast for 3 days and found hyperopic shifts (2.01 D) with changes in luminance contrast and myopic shifts with changes in color contrast. Rucker et al. (2015) determined that the reduction in eye length was most pronounced with exposure to high temporal frequencies (5–10 Hz), confirming earlier research (Gottlieb & Wallman, 1987; Schwahn & Schaeffel, 1997), and that the more myopically defocused blue component of the light source provided protection against increases in eye growth at low temporal frequencies with luminance flicker. These results have confirmed that the eye utilizes signals arising from temporally-sensitive changes in luminance and color contrast to determine the emmetropization response.

### *1.2. Parasympathetic and sympathetic control of color and luminance pathways*

The molecular pathways for these color and luminance signals are unknown. One possibility is that these light signals activate the parasympathetic and sympathetic nervous systems. The neurotransmitter acetylcholine (ACh) is released from parasympathetic axon terminals that innervate the ciliary body, iris, smooth muscle in the vasculature, but also from intrinsic interneurons in the retina. There are two categories of acetylcholine receptors: nicotinic (ionotropic; nAChR) and muscarinic (metabotropic; mAChR), which are coupled to heterotrimeric G-proteins (review: Nathanson, 1987). Atropine, which has been proposed as a treatment for myopia because of its effect in reducing eye growth, is a non-selective antimuscarinic. In mammals, five muscarinic receptor subtypes, M1 through M5, are present in the human eye (review: Mitchelson, 2012). Of these receptor types, the M3 receptor is the most predominant receptor type in human iris sphincter, ciliary body (causing accommodation), retina and sclera (Collison, Coleman, James, Carey, & Duncan, 2000; Gil, Krauss, Bogardus, & WoldeMussie, 1997; Ishizaka et al., 1998; Matsumoto, Yorio, DeSantis, & Pang, 1994; Pang, Matsumoto, Tamm, & DeSantis, 1994) with small amounts of M1, M4 and M5 in the iris sphincter and ciliary body (also M2) Mitchelson, 2012. There are also reports of mAChRs being expressed in the human RPE (Osborne, FitzGibbon, & Schwartz, 1991) and lens (Williams, Duncan, Riach, & Webb, 1993) with mainly M1 receptors in native human lens epithelium (Collison et al., 2000) and acetylcholinesterase on the lens surface (Michon & Kinoshita, 1968). Muscarinic receptors are found throughout the retina on amacrine, bipolar, horizontal and ganglion cells, though the only cholinergic cells in the adult retina are the starburst amacrine cells (Fischer, McKinnon, Nathanson, & Stell, 1998; McBrien, Jobling, Truong, Cottriall, & Gentle, 2009; Strang, Renna, Amthor, & Keyser, 2010; Townes-Anderson & Vogt, 1989; Yamada et al., 2003).

With regard to the chick animal model of myopia, four avian mAChR subtypes have been characterized: cm2 (Tietje & Nathanson, 1991), cm3 (Gadbut & Galper, 1994) cm4 (Tietje, Goldman, & Nathanson, 1990) and cm5 (Creason, Tietje, & Nathanson, 2000). Fischer et al. (1998) reported localization of three of the different isoforms of mAChRs (cm2–cm4) subtypes in the chick eye, in the retina, choroid, retina pigment epithelium (RPE) and ciliary body. It is important to note that chicks differ from mammals in that only nicotinic receptors are involved in accommodation (McBrien, Moghaddam, New, & Williams, 1993) and thus in chicks accommodation should not be affected by atropine.

In the human eye, the sympathetic nervous system innervates the ciliary muscle, ciliary epithelium, iris dilator muscle and smooth muscle of the vasculature. Innervation occurs through the action of the neurotransmitter noradrenaline on two subclasses of post-synaptic adrenergic receptor types: α- and β-adrenoceptors (review: Chen, Schmid, & Brown, 2003). Timolol maleate, which has been used as a clinical treatment for glaucoma since the late 70s, is a non-selective β-adrenoceptor antagonist (Airaksinen, Saari, Tiainen, & Jaanio, 1979). α-Adrenoceptors consist of two subtypes α1 and α2, which can be further subdivided into α2A, α2B and α2C subtypes (Regan & Cotecchia, 1992). Stimulation of α-adrenoceptors can regulate contraction of the iris dilator muscle (mydriasis) (van Alphen, 1976) and relaxation of the ciliary body (Garner, Brown, Baker, & Colgan, 1983; Zetterstrom, 1988). β-Adrenoceptors consist of two subtypes, β-1 and β-2. β-1 receptors are mainly found in cardiac tissues, but they also make up 10% of the receptors in human iris and ciliary body (Wax & Molinoff, 1987). Most of the receptors in the ciliary body are of the β-2 receptor subtype (Wax & Molinoff, 1987) and stimulation causes muscle relaxation. In addition, β-2 receptors control secretion from the non-pigmented ciliary epithelium, and blockade of these receptors by timolol reduces aqueous production (Zimmerman & Kaufman, 1977) and thus intraocular pressure (IOP). Many studies have reported that IOP readings are higher in human myopes than emmetropes (David, Zangwill, Tessler, & Yassur, 1985; Jensen, 1992; Maurice & Mushin, 1966; Parssinen, 1990; Quinn, Berlin, Young, Ziylan, & Stone, 1995), although the differences are small (2 mmHg) and not predictive of future myopia development (Goss & Caffey, 1999).

It is well established in the accommodation literature that dual excitatory parasympathetic and inhibitory sympathetic innervation to the ciliary muscle occurs (Toates, 1972; Tornqvist, 1967), though sympathetic innervation is much weaker (<−2D) and slower (maximal effect after 10–40 s) (Tornqvist, 1966). McBrien and Millodot (1986) suggested that late-onset myopes, with a reduced dioptric level of tonic accommodation, indicative of decreased parasympathetic tone, have a related decrease in inhibitory sympathetic tone. Furthermore, Gilmartin & Bullimore found that sympathetic blockade increases the decay time for accommodation after periods of extended near work (Gilmartin & Bullimore, 1987), particularly in late-onset myopes at high stimulus levels (5D) (Gilmartin & Bullimore, 1991). The authors' hypothesis that late-onset myopia may result from a deficit of the sympathetic nervous system has received considerable support (Chen et al., 2003; Ciuffreda & Lee, 2002; Ciuffreda & Wallis, 1998; Culhane, Winn, & Gilmartin, 1999).

In this study, we analyzed the effect of the non-selective parasympathetic antagonist atropine and the non-selective β-adrenergic receptor blocker timolol on the parasympathetic and sympathetic nervous systems' emmetropization responses to color and luminance flicker. We predicted that luminance and color stimulation may preferentially stimulate one or other of the autonomic nerve pathways, since exposure to high-frequency luminance flicker has been associated with a reduction in eye growth similar to that found with atropine.

## 2. Methods

### *2.1. Subjects*

Subjects were white leghorn chicks (*Gallus gallus domesticus*) Cornell K strain (Cornell University, Ithaca, NY), hatched in an incubator and raised in temperature-controlled brooders. Upon