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The absence of attenuating effect of red light exposure on pre-existing melanopsin-driven post-illumination pupil response ☆

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ABSTRACT

It has been proposed that after activation by blue light, activated melanopsin is converted back to its resting state by long wavelength red light exposure, a putative mechanism of melanopsin chromophore recovery *in vivo*. We tested this hypothesis by investigating whether red light attenuates the ongoing post-illumination pupil response (PIPR) induced by melanopsin-activating blue light. Pupillary light responses were tested using "Blue + Red" double flashes and "Blue Only" single flash stimuli in 10 visually normal subjects. For "Blue + Red" conditions, PIPR was induced with an intense blue flash, followed by experimental red light exposure of variable intensity and duration (Experiment 1) immediately or 9 s after the offset of the blue flash (Experiment 2). For "Blue Only" conditions, only the PIPR-inducing blue stimuli were presented (reference condition). PIPR was defined as the mean pupil size from 10 to 30 s (Experiment 1) and from 25 to 60 s (Experiment 2) after the offset of blue light stimuli. The results showed that PIPR from "Blue + Red" conditions did not differ significantly from those of "Blue Only" conditions ($p = 0.55$) in Experiment 1. The two stimulation conditions also did not differ in Experiment 2 ($p = 0.38$). We therefore conclude that red light exposure does not alter the time course of PIPR induced by blue light. This finding does not support the hypothesis that long wavelength red light reverses activated melanopsin; rather it lends support to the hypothesis that the wavelengths of stimuli driving both the forward and backward reactions of melanopsin may be similar.

1. Introduction

Intrinsically photosensitive retinal ganglion cells (ipRGCs) are a third class of photoreceptors in the retina (Berson, Dunn, & Takao, 2002; Hattar, Liao, Takao, Berson, & Yau, 2002). They contain melanopsin (Provencio, Jiang, Willem, Hayes, & Rollag, 1998; Provencio et al., 2000), a photopigment that mediates a depolarizing phototransduction cascade in ipRGCs, allowing these cells to fire action potentials on their own upon light stimulation (Berson et al., 2002; Hattar et al., 2002). Melanopsin-mediated ipRGC photoactivity provides sustained coding of ambient light irradiance for circadian rhythm photoentrainment and tonic pupil size regulation (Do & Yau, 2010; Lucas, 2013).

Much like rhodopsin in rods and photopsins in cones, melanopsin is also a vitamin A-based photopigment that employs 11-*cis*-retinal as its chromophore (Provencio et al., 1998). After absorbing a photon, 11-*cis*-retinal is photoisomerized into all-*trans*-retinal, causing conformational changes in the opsin, and subsequently triggering the phototransduction cascade in the photoreceptor (Ebrey & Koutalos, 2001). The activated chromophore, all-*trans*-retinal, must be converted back to 11-*cis*-retinal for the photoreceptor to regain its photosensitivity. Because of the close proximity to the underlying retinal pigmented epithelium (RPE), all-*trans*-retinal leaves rods and cones, and is converted back to 11-*cis*-retinal within the RPE. IpRGCs, however, are located in the inner retina distal from the RPE. Thus, the mechanism by which melanopsin-containing ipRGCs recycle their melanopsin chromophore without the close proximity to RPE has been a topic of active research. There is growing evidence (Matsuyama, Yamashita, Imamoto, & Shichida, 2012; Mure, Rieux, Hattar, & Cooper, 2007; Mure et al., 2009; Rollag, 2008; Teikari, 2012; Zhu et al., 2007) suggesting that melanopsin is capable of functioning as a photo-switchable opsin, that is, the isomerized chromophore

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(all-*trans*-retinal) does not leave the ipRGC, but instead remains attached stably to activated melanopsin (*meta*-melanopsin, M-state), and is converted back to the 11-*cis*-retinal conformation (resting state melanopsin, R-state) by subsequent absorption of light, the spectral sensitivity of which is controversial (see below).

While there is consensus that melanopsin activation (the forward reaction) is selectively sensitive to short wavelength light, with maximum absorption at around 480 nm (i.e., blue light) Berson et al., 2002; Do & Yau, 2010; Mure et al., 2009; Dacey et al., 2005; Do et al., 2009; Gamlin et al., 2007, conflicting evidence exists regarding the spectral sensitivity of melanopsin photo-regeneration (the reverse reaction) (Matsuyama et al., 2012; Mawad & Van Gelder, 2008; Mure et al., 2007, 2009; Rollag, 2008; Teikari, 2012; Zhu et al., 2007). Mure and co-workers (Mure et al., 2007, 2009) showed that pre-exposure to long wavelength red light potentiates the photo response of ipRGCs to short wavelength light (as measured by the spiking of neurons in the suprachiasmatic nucleus which mediates the circadian rhythm, the pupillary light reflex, and the negative masking effect), a feature of bistable opsins. They reported that the putative spectral sensitivity of *meta*-melanopsin is red-shifted, with maximal absorption at 587 nm (Mure et al., 2009). However, a similar experimental paradigm failed to potentiate ipRGC firing *in vitro*, arguing against this "blue forward, red reverse" hypothesis (Mawad & Van Gelder, 2008). Furthermore, in a recent photochemical study using purified rat melanopsin protein, Matsuyama and co-workers (Matsuyama et al., 2012) showed that melanopsin and *meta*-melanopsin have essentially overlapping spectral sensitivity, with both responses peaking within a narrow range of blue light (467 vs 476 nm). In the same study, a new melanopsin state containing 7-*cis* retinal (extra-melanopsin, E-state) with peak absorption wavelength at 446 nm was also discovered, leading to the idea of melanopsin "tri-stability" (Matsuyama et al., 2012). This idea was further supported by cellular electrophysiology evidence from mice ipRGCs that there are three photo-switchable states of melanopsin—two silent states (R- and E-state) and one signalling state (M-state)—and that the photo-equilibration among silent and signalling states is sensitive to short wavelength (blue) light only Emanuel & Do, 2015.

After being activated by an intense light exposure near its maximum absorption wavelength, a photoreceptor that employs a bistable opsin often exhibits a prolonged depolarizing after-potential (PDA) in the dark Hardie & Postma, 2008; Ashby & Schaeffel, 2010; Stone, Pardue, Iuvone, & Khurana, 2013. This electrophysiological property gives rise to another classic experimental paradigm to investigate opsin bistability in which a PDA is induced first by stimulating the photoreceptor with a pulse of intense light near its optimal excitation wavelength, followed by a second intense light exposure at a longer wavelength to determine if the initial PDA can be attenuated (Ashby & Schaeffel, 2010; Cohen, Peleg, Belkin, Polat, & Solomon, 2012). This paradigm has been used extensively in the investigation of opsin bistability in invertebrate photoreceptors (Ashby & Schaeffel, 2010), but it has not been tested *in vivo* in humans.

A number of studies have demonstrated that melanopsin-containing ipRGCs indeed show a PDA after exposure to intense melanopsin-activating blue light (Berson et al., 2002; Dacey et al., 2004, 2005; Hattar et al., 2002), leading to a sustained pupil constriction beyond the offset of light stimulus (Gamlin et al., 2007), which was thought to be a consequence of melanopsin bistability (Zhu et al., 2007; Rollag, 2008). This so-called post-illumination pupil response (PIPR) has been widely accepted as an *in vivo* index of the melanopsin-driven intrinsic ipRGC photo response (Feigl, Mattes, Thomas, & Zele, 2011; Herbst, Sander, Milea, Lund-Andersen, & Kawasaki, 2011; Herbst et al., 2012, 2013; Kankipati, Girkin, & Gamlin, 2010, 2011; Kawasaki, Crippa, Kardon, Leon, & Hamel, 2012; Kawasaki, Munier, Leon, & Kardon, 2012; Lei, Goltz, Chandrakumar, & Wong, 2014, 2015; Münch, Léon, Crippa, & Kawasaki, 2012; Nissen, Sander, & Lund-Andersen 2011; Nissen et al., 2014; Park et al., 2011; Roecklein et al., 2013). This phenomenon raises an intriguing question: can PIPR be reversed by red light exposure? Based on pre-existing data, it can be hypothesized that if the spectral sensitivity of *meta*-melanopsin is red-shifted, red light exposure will convert the signalling *meta*-melanopsin back to silent state and therefore attenuate the pre-existing PIPR, whereas if the spectral sensitivity of *meta*-melanopsin is similar to that of silent melanopsin and extra-melanopsin, red light exposure will have no effect on pre-existing PIPR. To provide evidence regarding the *in vivo* mechanism of melanopsin regeneration, we conducted two *in vivo* experiments with human subjects to test the effect of long wavelength red light exposure on pre-existing PIPR by presenting red light stimuli of variable intensity and duration at different time points during blue light-induced PIPR.

2. Methods

2.1. Participants

Ten visually-normal participants who had normal or corrected-to-normal vision (0.00 logMAR or better with ETDRS charts) participated in Experiments 1 and 2 (not all individuals participated in both experiments). There were 6 females and 4 males (mean age 31.5 years, age range 20–57 years) in Experiment 1, and 7 females and 3 males (mean age 32.1 years, age range 20–57 years) in Experiment 2. All participants underwent an eye exam, which include visual acuity, eye alignment and movement tests, colour vision assessment (HRR test, Richmond Products, NM, USA), intraocular pressure assessment using slit-lamp tonometry, slit-lamp assessment of iris structures, anterior chamber angle and a non-dilated fundus exam. The study was approved by the Research Ethics Board at The Hospital for Sick Children. All the procedures adhered to the guidelines of the Declaration of Helsinki. Written informed consent was obtained from each participant.

2.2. Apparatus and testing protocols

Red (640 ± 10 nm) and blue (470 ± 17 nm) monochromatic full field light stimuli were presented with a Ganzfeld bowl (Espion V5 system with the ColorDome LED full-field stimulator; Diagnosys LLC, Lowell, MA). Pupil responses were recorded using an infrared video-based spectacle frame-mounted eye tracker (Arrington Research, Scottsdale, AZ). The configuration and specifications of our chromatic pupillometry system have been described in detail previously (Lei et al., 2014, 2015).

All participants were exposed to an indoor laboratory environment with ambient lighting levels ranging from 80 to 400 lx (3000 K to 4000 K colour temperature) for at least 2 h prior to the experiment. During the pupillometry recordings, participants were seated in a quiet darkened room (0 lx) with their head resting on a chinrest, with the forehead touching the upper edge of the Ganzfeld bowl opening to receive full-field chromatic stimulation.

2.2.1. Experiment 1

Experiment 1 was designed to investigate whether PIPR can be attenuated by presenting red light *immediately* following the melanopsin-activating blue light. It was conducted monocularly, with one eye patched, while the PIPR was stimulated and recorded for the fellow eye during a "Blue Only" control condition and a "Blue + Red" experimental condition. In the "Blue Only" condition, PIPR was induced with a flash of a 400 cd/m^2 blue light