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Evidence for an impact of melanopsin activation on unique white perception

DINGCAI CAO^{1,*}, ADAM CHANG¹, and SHAOYAN GAI^{1,2}

¹Department of Ophthalmology & Visual Sciences, University of Illinois at Chicago, Chicago, Illinois 60612, USA

²Key Laboratory of Measurement and Control for Complex System of Ministry of Education, Southeast University, Nanjing, Jiangsu 210096, China

Abstract

Current models of human color vision only consider cone inputs at photopic light levels, yet it is unclear whether the recently discovered melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) contribute to color perception. Using a lab-made five-primary photostimulator that can independently control the stimulations of rods, cones, and ipRGCs in human retina, we determined the observer's unique white perception, an equilibrium point for signals arising from the opponent mechanisms of color vision, under different levels of melanopsin activation. We found changing melanopsin activation levels shifts the equilibrium point in the chromatic pathways. Our results suggest potential evidence for an impact of melanopsin activation on unique white perception and the existing color vision model for the periphery may need to be revised by incorporating melanopsin signaling.

OCIS codes:

(330.1690) Color; (330.5310) Vision - photoreceptors; (330.1720) Color vision

1. INTRODUCTION

Human color vision is considered to arise from two chromatic pathways, including the parvocellular (PC) and koniocellular (KC) pathways [1]. The PC pathway differentiates the long-wavelength-sensitive (L-) versus middle-wavelength-sensitive (M-) cones to signal red/green information. The KC pathway takes the differences between the short-wavelength-sensitive (S-) cones and the sum of L- and M-cones to signal blue/yellow information. This view, however, is subject to challenge due to the discovery of melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) [2,3]. To date, research has shown that ipRGCs are important for several subconscious non-image-forming functions, such as circadian photoentrainment and the pupil light reflex [3–9]. However, ipRGCs also project to the lateral geniculate nucleus (LGN), which relays retinal visual information to the visual cortex [10,11], suggesting that melanopsin activation may contribute to conscious

*Corresponding author: dcao98@uic.edu.

visual perception. Indeed, cone metamers with higher melanopsin excitation are reliably judged as brighter than those with lower melanopsin excitation [12], and brightness estimations are resulted from a combined contribution from cone and melanopsin signaling [13]. Further, a recent functional magnetic resonance imaging (fMRI) study in humans shows that the visual cortex responses induced by melanopsin signals are associated with brightened visual percepts [14]. Yet, knowledge about the functional consequences of melanopsin activation in human color vision is limited.

Our principal component analyses, based on human five photoreceptor activations (melanopsin, rods, S-, M-, L-cones) with natural images, also suggested that melanopsin activation may contribute to the PC and KC pathways to alter color perception [15]. Several studies have attempted to assess the contribution of melanopsin activation to human color perception but with conflicting results. Brown *et al.* reported that melanopsin did not affect chromatic discrimination [12]. By contrast, Horiguchi *et al.* found that foveal chromatic discrimination data could be modeled by a trichromatic model, but peripheral chromatic discrimination data required the fourth photopigment, potentially melanopsin, compared with foveal data [16]. However, their study did not consider the intrusion of penumbral cones, which locate under the shadow of retinal blood vessels and have slightly different spectral sensitivity functions due to hemoglobin absorption [17]. In addition, their subjects did not show consistent results, with some of their subjects having discrimination affected by the fourth pigment along S-cone versus L- or M-cone planes (presumably mediated by the KC pathway). These contradictory findings may reflect the differences in methodologies as well as the experimental conditions.

In this study, we aimed to assess whether melanopsin activation in ipRGCs contributes to color perception by determining whether different melanopsin activation levels affected unique white perception, which is the experience of absence of hues, i.e., nor reddish, nor greenish, nor bluish, nor yellowish. Unique white perception is considered to arise from the equilibrium point for signals from the opponent mechanisms of color vision, either the equilibrium point from the two chromatic pathways (PC and KC pathways, corresponding to the two cardinal axes) at the physiological level or red/green and blue/yellow opponency at the perceptual level. We hypothesized that melanopsin activation could contribute to color perception, as manifested by a shift in unique white setting with different melanopsin activation levels.

2. METHODS

A. Observers

Three male subjects with normal corrected acuity participated in the experiment (S1, age 46 years; S2, age 34 years; and S3, age 37 years; S1 was the first author of this paper). All observers had normal color vision assessed by the Farnsworth–Munsell 100 Hue test and the Nagel anomaloscope. The study protocols were approved by the Institutional Review Board at the University of Illinois at Chicago and were in compliance with the Declaration of Helsinki. We obtained written consent for all subjects. All of the procedures were carried out in accordance with US government human subject protection guidelines and regulations.

B. Apparatus

We developed a five-primary Maxwellian-view photostimulator that is capable of independently controlling the excitation levels of the five different types of photoreceptors (S-cone, M-cone, L-cone, rod, and ipRGC) in the human retina using the silent substitution method [18]. The custom-made device has five different colored LEDs—"red," "amber," "green," "cyan," and "blue" (dominant wavelength: "red"—632 nm, "amber"—592 nm, "green"—540 nm, "cyan"—488 nm, "blue"—456 nm). The LEDs had little thermal shifts with different duty cycles, and they were sandwiched with interference filters to ensure stable spectral outputs. The lights from the LEDs travel through optic fibers before being combined in a spatial homogenizer and a diffuser, producing a highly uniform field. A field lens with a 2-mm artificial pupil was used to create a Maxwellian view. Light attenuation was achieved using calibrated neutral density filters positioned in front of the field lens. We developed custom software using Objective-C on a Mac computer in order to control the LED outputs.

The five-primary photostimulator allowed independent control of the stimulations of five types of photoreceptors (S-cones, M-cones, L-cones, rods, and melanopsin-containing ipRGCs) in a human retina, using a silent substitution method [19,20]. The cone excitations were computed based on the Smith–Pokorny cone fundamentals applied for the CIE 1964 10° Standard Observer [21]. The rod excitation (R) was computed based on the CIE 1951 scotopic luminosity function. The melanopsin-mediated ipRGC excitation (I) was computed according to the melanopsin spectral sensitivity function [22]. The spectral sensitivity functions of L-cones, M-cones, S-cones, rods, and melanopsin-containing ipRGCs were normalized in a similar fashion as the relative Troland space [23], such that 1) for an equal-energy-spectrum light at 1 Td, the excitations of rods, S-cones, and melanopsin-containing ipRGCs were all equal to 1 Td, while the excitations of L-cones were 0.667 Td and excitations of M-cones were 0.333 Td; and 2) the luminance is equal to the sum of the L- and M-cone excitations (i.e., $L + M$). In such a normalization, the relative photoreceptor excitation can be expressed as $l = L/(L + M)$, $s = S/(L + M)$, $r = R/(L + M)$, and $i = I/(L + M)$. Note $l = L/(L + M)$ and $s = S/(L + M)$ are two cardinal axes in a MacLeod & Boynton equiluminant cone chromaticity space [24], corresponding to the PC and KC pathways, respectively [25,26].

C. Calibration

The instrument was calibrated in three steps: 1) primary LED spectral power distribution measurement using a PR-670 spectroradiometer; 2) LED linearization for 4096 digital levels using an International Light ILT1700 current meter; 3) LED photopic illuminance measurement using an EG&G 550 radiometer/photometer. Details of the instrument and calibration procedures can be found in our previous publication [18,27]. After physical calibration, we conducted an observer calibration using heterochromatic flicker photometry (HFP) at 15 Hz to establish equiluminance of the primaries for each observer. We conducted additional measurements to confirm the observer calibration and photoreceptor isolation. A 500 ms, 18% Weber contrast rod pulse (no change in the melanopsin or cone excitations) at a 5 Td mesopic adaptation level was invisible after photopigment bleach but was highly conspicuous after dark adaptation. The rod percept was matched by cone excitations with a

decrease in $L/[L + M]$, increase in $S/[L + M]$ and an increase in $[L + M]$, consistent with our previous findings [28,29].

D. Stimuli

The stimuli were a 30° circular field with the central 10.5° blocked to remove the effect of macular pigment's selective absorption. A small hole (<1 min arc) was created in the light blocker center to serve as the fixation; therefore, the fixation had the same light level as the annulus. Experiments were conducted at three photopic light levels, including 2000, 6000, and 10,000 Td. At each light level, unique white was measured with two conditions, "Mel-High" and "Mel-Low". The relative melanopsin excitations [$i = I/(L + M)$] were 1.05 with the "Mel-High" condition and 0.95 with the "Mel-Low" condition, such that the "Mel-High" condition had 10.5% higher melanopsin activation level than the "Mel-Low" condition. The relative rod excitation [$r = R/(L + M)$] was fixed at 0.98 for all experiments. Note the stimuli were set to equate cone excitations in the periphery based on CIE Standard 10° observer for the "Mel-Low" and "Mel-High" conditions, the cone excitations were not equal in fovea for the fixation lights. The chromaticity difference between the two conditions in the fixation was 0.8% in $I = L/(L + M)$ and 4.7% in $s = S/(L + M)$. However, for our small fixation, the observers could not tell any color difference in the fixation lights between the conditions; therefore, the findings in the study could not be caused by the unequal cone excitations in the fixation lights. In addition, as there was large distance between the fixation and the annulus, the impact of scatterlight from the fixation into the test area was minimal.

E. Procedure

The experiments were conducted in a dark room. During each trial of the experiments, the observers were asked to fixate a small fixation point in the center of the stimuli.

1. Unique White Setting Procedure—At the beginning of each trial, the stimulus was given a random chromaticity of $I (= L/(L + M))$ and $s (= S/(L + M))$ values near equal-energy white (EEW) ($I = 0.667$, $s = 1.00$). This randomization important, was as the starting point of the stimulus may have a short-term adaptation effect on the observer. During the experiment, the observer adjusted the I , s values of the stimulus using the upward, downward, left, and right buttons pad on a USB Logitech Precision game controller in order to achieve a white perception (i.e., non-reddish, non-greenish, non-bluish, and non-yellowish). Pressing upwards increased s , or koniocellular pathway excitation, causing the stimulus to look increasingly purple, a combination of a "blue" and "red" increase. Pressing downward decreased s , causing the stimulus to appear both more yellowish and more greenish. Pressing right or left increased or decreased I , causing the stimulus to become more reddish or more greenish, respectively. A separate button was designated to confirm unique settings. During the unique white setting, the rod and melanopsin excitations and luminance ($L + M$) were held constant throughout the observer's adjustment in the I and s directions.

For each melanopsin condition/illuminance combination, observers completed five trials at a block and completed eight blocks in total. Each trial took about 1–2 min with inter-trial-interval as 1 min. The order of melanopsin activation level was randomized at each light level. The experiment was performed over the course of several days.

2. Hue Scaling Procedure—Instead of asking the observers to adjust the l and s chromaticity to achieve unique white setting, we also the observers to reported hue and saturation of steady lights with cone chromaticity of EEW (i.e., $l = 0.667$, $s = 1.0$) with two identical melanopsin activation levels to the unique white setting experiment (the “Mel-High” and “Mel-Low” conditions). Subjects were instructed to report the perceived hue and saturation [30]. Hue was reported in the percentages of the four basic hues (Red, Green, Blue, Yellow), with the sum of the four basic hues to be 100%. Saturation was reported between 0%–100%. Subjects completed four trials at different times for each melanopsin condition/illuminance combination on different days.

3. RESULTS

For all three observers, the l values of the “Mel-High” condition were consistently higher than those of the “Mel-Low” conditions at all three light levels tested (Fig. 1, top panels, $p < 0.001$), suggesting the “Mel-High” condition was more greenish than the “Mel-Low” condition. On average, as melanopsin activation level increased by 10.5%, the l value of the unique white setting increased by 0.003 for S1, 0.005 for S2, and 0.003 for S3 for all three light levels, which were ~ 1.5 – 2.5 times of the l discrimination threshold (~ 0.002 [31]). However, the differences in the s values between the conditions were not statistically different (Fig. 1, bottom panels, although the “Mel-High” tended to have higher s values at 6000 Td and 10,000 Td for S1 and S3, respectively), probably due to large variability among the observations in this direction for unique white setting [32]. Subjective report of hue and saturation (i.e., hue scaling [30]) for steady lights with cone chromaticity of EEW (i.e., $l = 0.667$, $s = 1.00$) indicated that the “Mel-High” condition had a more greenish appearance associated with a much lower saturation than the “Mel-Low” condition (Fig. 2). The results for blueness/yellowness rating were not consistent, with two of the three observers (S1 and S3) reported that the “Mel-High” condition has higher yellowness rating than the “Mel-Low” condition.

4. DISCUSSIONS

Our results suggested that melanopsin activation level significantly shifted the equilibrium point in the l direction but not s direction. Anatomically, ipRGCs project to the LGN [10,11], thus melanopsin activation can potentially alter chromatic processing. Principal component analysis of photoreceptor excitations in natural images suggested that melanopsin activation contributes to the PC and KC pathways [15]. The data from our unique white settings indicated that melanopsin activity has a profound effect on the PC pathway; its contribution can be thought of as additive to the M-cone signal opposing the L-cone signal in the PC pathway [i.e., $L - (M + I)$](where “I” for melanopsin activation ipRGCs) to signal greenness and/or blueness. This photoreceptor combination pattern was also identified based on principal component analysis in the natural image statistics [15]. On the other hand, ipRGCs have S-OFF versus $L + M$ – ON response properties, based on primate physiology [10] or human pupil recordings [18,27,33], suggesting melanopsin activation may contribute to yellowness as opposed to S-cone contribution to color perception. Indeed, perceptual matching experiment indicated that melanopsin-mediated percept could be matched with increasing ($L + M$) and decreasing S stimulation [34]. In our

experiment, two of our three observers showed increased yellowness rating with a higher melanopsin activation. The same subjects tended to require higher s in unique white setting. Consistent with this, Spitschan *et al.* [14] reported melanopsin stimulation was “usually” but not “universally” associated with yellow-orange percept, although they could not rule out cone intrusion.

Our findings for melanopsin contributions to color vision are dependent on the success of silent substitution. For healthy observers with normal color vision, pre-receptor filtering differences are the largest sources of variation in the estimates of corneal photoreceptor spectral sensitivities of normal trichromats [35]. Our observer calibration procedure can minimize individual differences between participants and the theoretical standard observer $L + M$ cone luminous efficiency function. By doing so, each observer had a personalized estimate of their cone luminous efficiency that was used to normalize the relative photoreceptor excitations of the S-cones, rods, and melanopsin and therefore to improve cone silencing. This observed effect cannot be attributed to rod intrusion [28,29], as the conditions tested upon were set at photopic light levels and, additionally, the rod excitation was held constant for all experiments. Further, this effect cannot be explained by the intrusion of penumbral cones in the shadow of the retinal vasculature that receive a different quantal catch due to attenuation of the light stimulus by hemoglobin absorption [17]. We used steady lights to minimize the intrusion of penumbral cones, as the Purkinje tree (retinal blood vessel structure) is not visible with steady lights due to adaptation [17,36].

Taken together, our study showed that unique white perception could be affected by melanopsin activation level, suggesting melanopsin activation may contribute to color perception. As current human color vision models only consider cone inputs, our results indicate that the models may need to be revised by incorporating melanopsin signaling.

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Fig. 1.

Unique white I (top) and s (bottom) values (mean $\pm$ sem) as a function of retinal illuminance for three observers. ** $p < 0.01$; *** $p < 0.001$ from t-tests.

Fig. 2.

Hue scaling results. Top panels: the rated redness (positive values) or greenness (negative values). Middle panels: the rated blueness (positive values) or yellowness (negative values). Bottom panels: the rated saturation. ** $p < 0.01$; *** $p < 0.001$.