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Melatonin Regulation in Humans with Color Vision Deficiencies*

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ABSTRACT

Light can induce an acute suppression and/or circadian phase shift of plasma melatonin levels in subjects with normal color vision. It is not known whether this photic suppression requires an integrated response from all photoreceptors or from a specialized subset of photoreceptors. To determine whether normal cone photoreceptor systems are necessary for light-induced melatonin suppression, we tested whether color vision-deficient human subjects experience light-induced melatonin suppression. In 1 study, 14 red-green color vision-deficient subjects and 7 normal controls were exposed to a 90-min, 200-lux, white light stimulus from 0200–0330 h. Melatonin suppression was observed in the controls ($t = -7.04$; $P < 0.001$), all color vision-deficient subjects ($t = -4.76$; $P < 0.001$), protanopic observers ($t = -6.23$; $P < 0.005$), and deuteranopic observers ($t = -3.48$; $P < 0.05$), with no significant difference in the magnitude of suppression between groups. In a second study, 6 red/green color vision-deficient males and 6 controls were exposed to a broad band green light stimulus (120 nm with λ_{max} 507 nm; mean ± SEM, 305 ± 10 lux) or darkness from 0030–0100 h. Hourly melatonin profiles (2000–1000 h) were not significantly different in onset, offset, or duration between the two groups. Melatonin suppression was also observed after exposure to the green light source at 0100 h (color vision deficient: $t = -2.3$; df = 5; $P < 0.05$; controls: $t = -3.61$; df = 5; $P < 0.01$) and 0115 h (color vision deficient: $t = -2.74$; df = 5; $P < 0.05$; controls: $t = -3.57$; df = 5; $P < 0.01$). These findings suggest that a normal trichromatic visual system is not necessary for light-mediated neuroendocrine regulation. (*J Clin Endocrinol Metab* **81:** 2980–2985, 1996)

MELATONIN is an indole synthesized by the pineal gland and is secreted in a 24-h circadian pattern (1, 2). Characterizations of the daily variation in the melatonin rhythm have demonstrated that the hormone is secreted maximally during the nocturnal hours and minimally during the daylight hours. This pattern has been observed across mammalian species in both nocturnal and diurnal rodents and humans (3, 4).

Acute pulses of light during the normally dark nocturnal hours induce a suppression of melatonin secretion in mammals (5, 6) as well as in humans (7). Numerous studies have examined the relationship of stimulus intensity, wavelength, duration, and timing on light-induced melatonin suppression (8–14).

In addition to the acute effects on melatonin secretion, photic stimuli also entrain the circadian phase of the melatonin and other endogenous circadian rhythms (15–20). The effects of light on melatonin regulation are mediated by a neural pathway beginning with the retina and projecting to the suprachiasmatic nuclei via the retinohypothalamic tract, which, in turn, relays photic information to the pineal gland and other brain nuclei (21–25). Although photoreceptors for both circadian and visual functions are located in the retina, the neural pathway for circadian modulation is distinct from the visual neural projections to the thalamus. Observations that visual blindness in humans caused by either retinal destruction or an interruption of the retinohypothalamic tract can result in a loss of entrained circadian rhythmicity (26–28) suggest that photic transduction of melatonin regulation lies within the retina. Recent work, however, has identified retinally blind individuals with no conscious light perception who demonstrate entrainment of melatonin secretory rhythms (29). Given these findings, although light transduction in the visual system is a clear function of the cone and rod photopigments, the photoreceptor mechanism for circadian and neuroendocrine regulation remains unclear (30–32).

The individual with a color vision deficiency is a viable model for the assessment of the role of color vision in human circadian phototransduction because of an inherent difference in spectral color perception. In particular, specific deficiencies in color vision perception are based on underlying differences in cone photoreceptor pigment absorption spectra (33). In the case of protanopia, affected individuals behave as though there is a lack of the long wavelength-sensitive or red cone photoreceptor system, and in deuteranopia, individuals behave as if there is a lack in the middle wavelength-

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sensitive or green cone photoreceptor system. The following studies sought to examine whether red-green vision deficiencies might alter light-mediated melatonin suppression and the short term regulation of the melatonin rhythm. The results from these independent studies suggest that normal color vision is not necessary for light-mediated circadian and neuroendocrine regulation when using a broad band white light stimulus or a moderate band width (120 nm) light stimulus. These studies were conducted by separate research groups in different laboratories and in different countries, unaware of each other's protocol until the completion of each study.

Experimental Subjects

Exp 1

Both males (n = 18; mean age ± SEM, 24.2 ± 0.7 yr) and females (n = 3; age ± SEM, 27.3 ± 3.4 yr) recruited from local student populations in the Philadelphia area participated in this study. All reported being free of medication and having normal sleep patterns before beginning the experimental protocol. Subjects signed an informed consent approved by the institutional review board of Thomas Jefferson University before participation. Subjects were asked to refrain from alcohol and caffeine after their evening meal on the nights of experimental observation.

Screening resulted in the identification of 14 subjects with deficiencies in color vision (age, 25.3 ± 1.0 yr) and 7 controls with normal color vision (age, 23.4 ± 0.6 yr). Further characterization of the color vision-deficient subjects revealed 5 protanopic observers, 6 deuteranopic observers, 1 unspecified in deficiency, and 3 anomalous trichromatic observers. All color-deficient subjects exhibited color confusion along the red-green color vision axis.

Exp 2

Twelve healthy male volunteers (age, 32.7 ± 3.4 yr) participated in this study. All reported being free of medication and had normal sleep patterns before beginning the experimental protocol. All subjects signed an informed consent approved by the ethics committee of the South West Surrey Health Authority before participation.

The screening protocol identified six red/green color vision-deficient subjects. Age-matched controls with normal trichromatic color vision were then identified and recruited.

Two days before each experimental session, volunteers were asked to retire to bed at 2300 h and arise at 0700 h. Subjects were also asked to refrain from heavy exercise, alcohol, and caffeine consumption 12 h before the experiment.

Materials and Methods

Exp 1

Before acceptance into the experiment, all subjects underwent a three-part screening procedure. Each subject had his/her color vision tested with either Ishihara's Test for Color Vision (Kanehara Co., Tokyo, Japan) or the Standard Pseudoisochromatic Plates (Igaku-Shoin, Tokyo, Japan), the Farnsworth-Munsell Dichotomous Test D-15 (The Psychological Corp., New York, NY), and the Farnsworth-Munsell 100 Hue Test (Munsell Color, Macbeth/Kollmorgen Corp., Baltimore, MD). Subjects who passed the pseudoisochromatic plates were considered to have normal color vision and were used as control subjects. All subjects were further evaluated with the D-15 and the FM 100-Hue Test to determine the nature of any specific color confusion axis and to quantify discrimination deficiency by the 100-hue error score. Unpaired Student's two-tailed *t* test was employed to determine significant differences in error scores between the color-deficient and control groups. Color-deficient subjects were further evaluated using a Nagel anomaloscope to assess whether they were dichromatic or anomalous trichromatic observers, as evidenced by the ability of the dichromatic individual to match pure red or pure green with the reference yellow of this test.

After screening, each subject participated in the 2-night experimental protocol. On 1 night, subjects were exposed to uninterrupted darkness, whereas on the other night, subjects were exposed to a light stimulus. The order of the two experimental conditions was randomly assigned across subjects. On each test night, subjects arrived at the test facility at 2345 h. At approximately 0015 h, one drop of 0.5% cyclopentolate HCl ophthalmic solution, an anticholinergic mydriatic agent (Cyclogyl, Alcon Laboratories, Fort Worth, TX), was placed in the eyes of each subject. After cyclopentolate treatment, subjects were blindfolded, seated in a dark room (<5 lux), and asked to remain seated until 0200 h. At 0200 h, 10–14 mL blood were drawn into ethylenediamine tetraacetate-treated tubes from each subject by antecubital venipuncture.

On the predesignated light exposure night, subjects removed their blindfolds after the 0200 h sample and were seated in a modified Goldmann perimeter. As previously described (34), the dome was illuminated with a polychromatic (white) stimulus provided by a quartz halogen incandescent lamp (model EJA, General Electric Corp., Cleveland, OH), mounted in a source (FO-150-DPHM, Chiu Technical Corp., Kings Park, NY) that was fitted with a fiber-optic light guide (model SI-40–8, Chiu Technical) aimed at the top of the perimeter. Intensity was calibrated with an adjustable aperture at the light source to provide 200 lux of light incident to the subject's cornea. Illuminance was measured with an illuminance meter (Minolta, Osaka, Japan) at 0200 h and every 30 min thereafter during the course experiment. Illuminance was adjusted when necessary to maintain a constant level. During this exposure, the subjects were asked to keep their eyes open and their gazes fixed on a target in the back of the dome. The subject's gaze and pupillary dilation were monitored at 0200 h and every 30 min thereafter by means of a telescopic reticle mounted at the back of the dome. After a 90-min light exposure, a second blood sample was drawn at 0330 h. After sampling was complete, the subjects were discharged.

On the control (dark exposure) night, blood samples were collected at the same time points; however, subjects remained seated upright and blindfolded for the duration of the experimental period. The 2 study nights were separated by at least 1 week.

Blood samples collected during the study were separated at 4 C by centrifugation, stored at −20 C, and later assayed for melatonin content by RIA using a technique derived from that of Rollag and Niswender (6). Before RIA, chloroform extracts of the plasma samples were washed twice with 15 vol (3 mL) petroleum ether. The radioiodinated melatonin analog was prepared by adding 1 μmol 5-methoxytryptamine and 1 μmol tri-*N*-butylamine dissolved in 10 μL dioxane to 250 μCi (0.1 nmol) dry Bolton-Hunter reagent (New England Nuclear Corp., Boston, MA); the reaction was allowed to proceed for 10 min before electrophoretic separation of products. Assay results were not corrected for recovery (which has proven to be >95% in independent trials). The minimum detection limit of the assay was 0.5–2 pg/mL. Control samples containing 23 and 113 pg/mL melatonin gave interassay coefficients of variation of 8.8% and 10.5%, respectively.

As previously described (35), using the plasma melatonin concentrations associated with each time point, change scores for each experimental condition were calculated by subtracting the 0200 h melatonin level from the 0330 h melatonin level, yielding an index of melatonin change during the 90-min test period. By this convention, a negative change score indicated melatonin suppression, whereas a positive change score reflected a rise in the plasma melatonin concentration. Subtraction of the dark exposure change score from the light exposure change score yielded a control-adjusted index of melatonin suppression. Paired two-tailed Student's *t* tests were employed to determine significant differences between the dark exposure and light exposure change scores within subjects. ANOVA performed on the control adjusted change scores was used to test for significant differences between subject groups in the magnitude of plasma melatonin suppression.

Exp 2

Before acceptance into this experiment, all subjects were screened for color vision abnormalities using Ishihara's Test for Color Vision (Kanehara Co.). Subjects who failed the color plate test were identified as color deficient. Age-matched controls were subsequently recruited and submitted to the Ishihara test.

Subjects were studied on two occasions, separated by an interval of at least 1 week. On each experimental night, subjects were given a standard meal upon arrival at the Investigation Unit (University of Surrey). Between 1900–2000 h, an indwelling cannula was inserted into

a superficial radial vein. From 1900–2330 h, subjects experienced room lighting (150–250 lux). At 2330 h, subjects retired to bed in darkness (<1 lux).

On the predesignated light exposure night, subjects were awakened at 0030 h and seated before a light box fitted with fluorescent lamps (Vita-Lite, Duro-Test Corp., North Bergen, NJ) providing broad spectrum white light (correlated color temperature, 5500 Kelvin; color rendering index, 91). The adherence of a filter [no. 424, Dark Green (Primary), Strand Filters, London, UK] across the source produced green light stimulus (λ_{max}, 507 nm; range, 470–590 nm). Illuminance was measured with an illuminance meter (Minolta). The range of illuminance produced was 250–370 lux, with a mean ± SEM of 305 ± 10 lux. Subjects were seated approximately 1.0 m from the source and instructed to look at a spot in the center of the light source. After a 30-min exposure, volunteers returned to bed until 0700 h, after which they were exposed to both indoor and outdoor illumination (400–5000 lux) until 1000 h. On the predetermined dark exposure night, subjects were similarly awakened at 0030 h and sat in darkness (<1 lux) for the 30-min test period. Subjects returned to bed after the dark exposure until 0700 h.

Blood samples were collected hourly from 2000–1000 h as well as at 2330, 0015, 0030, 0045, 0115, and 0130 h. After samples were separated by centrifugation, plasma was stored at −20 C until analysis. Plasma melatonin levels were measured by direct RIA, as described by Fraser *et al.* (36). The minimum detection limit of the assay was 5 pg/mL. Plasma pools containing 119 and 226 pg/mL melatonin gave interassay coefficients of variation of 7.2% and 4.4%, respectively.

Paired one-tailed Student's *t* tests were employed to determine significant differences between the dark and green light exposures in both sets of subjects at the time points immediately after the exposure period (10). ANOVA performed on the control adjusted change scores was used to test for significant differences between subject groups in the magnitude of plasma melatonin suppression. A plasma melatonin concentration of 10 pg/mL was chosen as the reference value to determine onset and offset times as well as the duration of a subject's nocturnal melatonin production. Onset and offset clock times were expressed as decimal values. Acrophases (hours and minutes) were calculated for each individual's melatonin profile by the method of least squares using a cosine curve-fitting program (37).

Results

Exp 1

The mean FM100 score ± SEM for the control subjects was 46 ± 12.9, and that for the color vision-deficient subjects was 231 ± 24.5. These scores varied significantly between subject groups ($t = -5.13$; df = 19; $P < 0.001$) and thus provided a quantifiable assessment of the magnitude of color vision deficiency.

After the 90-min exposure to 200-lux, full-field white light, a significant suppression of the plasma melatonin concentration was observed in both the control group ($t = -7.04$; df = 6; $P < 0.001$) and the color vision-deficient group [$t = -4.76$; df = 13; $P < 0.001$; with the color vision-deficient subject group including all (n = 14) color vision-deficient subjects identified]. Subdivision of the color vision-deficient subjects into diagnostic grouping allowed for the assessment of melatonin changes in subjects with specific known photoreceptive system deficiencies. Figure 1 illustrates the plasma melatonin change scores (in picograms per mL) for the two color vision-deficient diagnostic subgroups and the control subjects. Significant plasma melatonin suppression was observed in the protanopic observers ($t = -6.23$; df = 4; $P < 0.005$) and the deuteranopic observers ($t = -3.48$; df = 5; $P < 0.05$). Figure 2 demonstrates the comparison of the control adjusted melatonin change scores for each subject group. ANOVA yielded no significant difference in the magnitude of plasma melatonin suppression with the light stimulus between subject groups [F(2,17) = 1.7; $P = 0.22$].

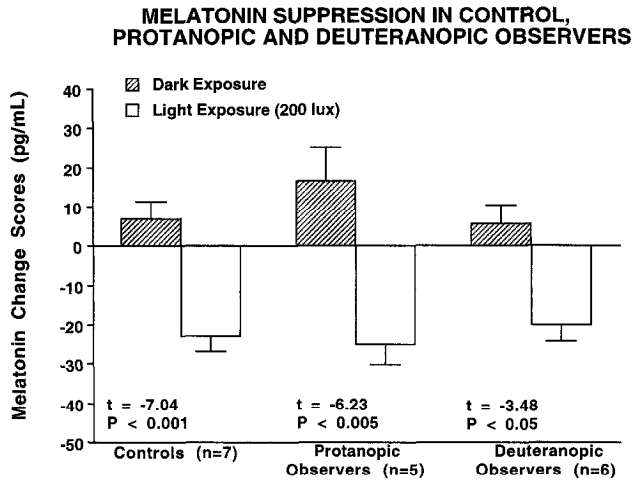

FIG. 1. Changes in plasma melatonin concentration in normal subjects (n = 7), protanopic observers (n = 5), and deuteranopic observers (n = 6) after a 90-min darkness exposure or a 90-min, full-field, white light exposure (200 lux). Subjects were exposed to both experimental conditions on 2 separate nights while their pupils were pharmacologically dilated. Mean preexposure plasma melatonin concentrations were 52.0 ± 4.5 pg/mL for the control group, 56.4 ± 9.6 pg/mL for the protanopic observers, and 42.2 ± 9.0 pg/mL for the deuteranopic observers. *Error bars* represent the SEM.

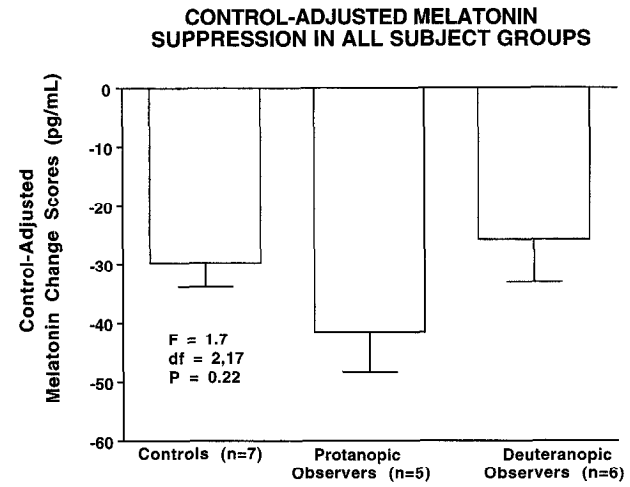

FIG. 2. Control adjusted changes in plasma melatonin concentration for the control group, protanopic observers, and deuteranopic observers. Subjects were exposed to 90 min of darkness and 90 min of full-field, white light (200 lux) on 2 separate nights while their pupils were pharmacologically dilated. No significant differences were observed between these groups. *Error bars* represent the SEM.

Exp 2

Figure 3 depicts the melatonin profiles ± SEM of both the control and color vision-deficient subjects between 0000–0200 h. Exposure to the 507-nm green light stimulus at 305 lux from 0030–0100 h significantly suppressed plasma melatonin concentrations in both groups at 0100 h (color vision deficient: $t = -2.3$; df = 5; $P < 0.05$; controls; $t = -3.61$; df = 5, $P < 0.01$) and 01:15 h (color vision deficient: $t = -2.74$; df = 5; $P < 0.05$; controls: $t = -3.57$; df = 5; $P < 0.01$). This figure

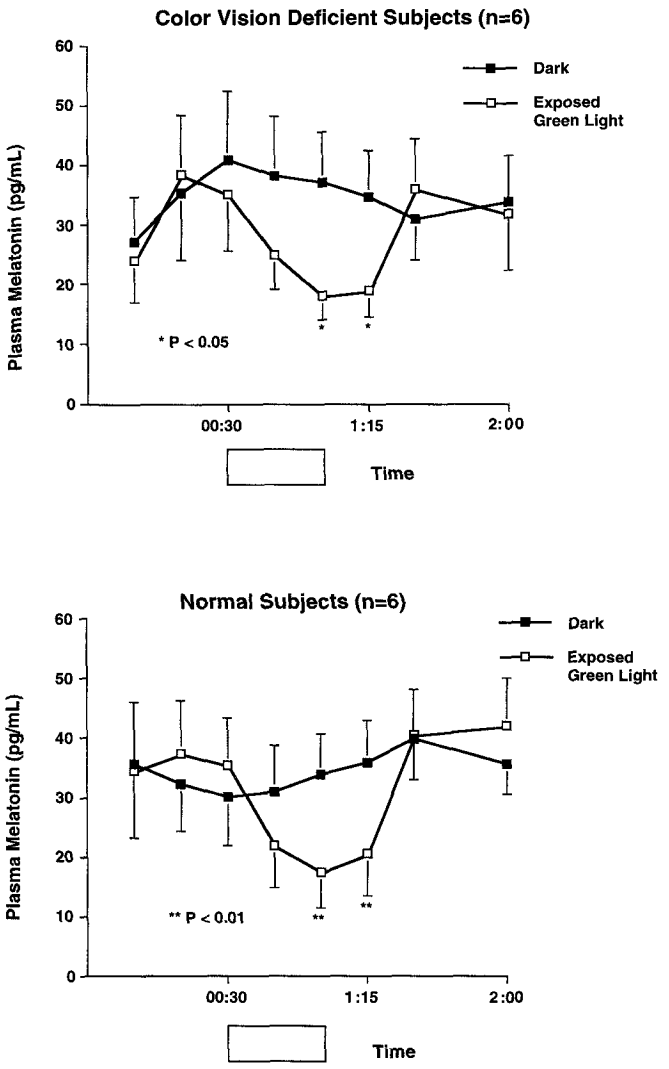

FIG. 3. Exposure to the green (λ_{max}, 507 nm; range, 470–590 nm) light stimulus (mean, 305 ± 10 lux; 0030–0100 h) significantly suppressed plasma melatonin concentrations at 0100 and 0115 h. *Error bars* represent the SEM.

also describes the melatonin profiles ± SEM of both the control and color vision-deficient subjects between 0000–0200 h. ANOVA yielded no significant difference in the magnitude of plasma melatonin suppression with the light stimulus between subject groups [$F(1,11) = 0.91$; $P = 0.36$]. Melatonin levels were significantly elevated on the night of light exposure (a rebound effect) at 0300, 0400, and 0500 h in the control group and at 0600 and 0700 h in the color vision-deficient group.

Analysis of the plasma melatonin rhythms of the control and color vision-deficient subjects was performed using a cosine curve-fitting program (37). In both groups after darkness exposure, the onset (color vision deficient, 22.9 ± 0.4 h; control, 23.4 ± 0.5 h), offset (color vision deficient, 7.2 ± 0.3 h; control, 7.3 ± 0.7 h), duration (color vision deficient, 8.2 ± 0.3 h; control, 7.9 ± 0.6 h), and acrophase (color vision deficient, 3.08 ± 0.14 h.min; control, 3.20 ± 0.35 h.min) times were similar. In addition, there were no significant changes in the onset, offset, or duration of the melatonin rhythms after light exposure.

Discussion

The experiments reported here represent a novel approach of examining light-mediated melatonin regulation in subjects with color vision deficiencies. These studies represent a collaboration between two research groups, each independently seeking to determine whether a functional loss in a cone photoreceptor system could affect light-mediated melatonin regulation. In one study, acute melatonin suppression was observed not only across all color vision-deficient subjects regardless of specific deficit grouping, but also within diagnostic subgroups (protanopia and deuteranopia). The magnitude of melatonin suppression with light in these color vision-deficient subjects was not significantly different from that in matched controls. In the other study, analysis of hourly profiles of the melatonin rhythm revealed no significant differences between normal trichromatic controls and color vision-deficient subjects in the onset, offset, or duration of the rhythm. In this same study, an acute suppression of melatonin secretion was observed 30 and 45 min after light exposure in both color vision-deficient and control subjects. These findings suggest that a normal trichromatic color perceptual system is not necessary for light-mediated melatonin regulation. Further, these findings suggest that perceptual anomalies in either the long wavelength (red) perceptual system or the middle wavelength (green) perceptual system may not affect light-induced melatonin suppression when stimulated by broad spectrum light.

Demonstration of light-induced melatonin suppression has been used in the past as an indicator that light input mechanisms to the general circadian system are intact. Recent work, however, has shown that there are different intensity-response relationships for light-induced melatonin suppression and light-induced phase shifting of wheel running circadian rhythms in hamsters (13). Thus, it is possible that the acute effects of light on melatonin suppression and the longer term effects of light on circadian regulation are mediated by different retinal and neural mechanisms. In the studies reported here, one experiment examines the acute effects of light on melatonin suppression, whereas the second experiment assesses both light-induced melatonin suppression and the short term effects of light on circadian features of the melatonin rhythm. The results of these independent experiments are consistent; a normal trichromatic visual system is not necessary for melatonin regulation. Neither experiment, however, attempts to characterize the longer term circadian effects of light on melatonin secretion in color vision-deficient individuals.

It must be emphasized that the isolation of color vision-deficient subjects was accomplished by a series of clinical tests that identified anomalous color perception (38). Although these deficiencies may be along the neural pathway projecting to the visual cortex or in the cortex itself, they probably lie within the retina as in the alteration or loss of a particular cone type or cone photopigment. This conclusion is supported by previous research into congenital color vision deficits using reflection densitometry of the retina,

which has determined quantitative differences in cone photopigments among color blind individuals and normal trichromats (39). Subsequent genetic studies suggested that anomalous gene expression results in shifts of the cone pigment absorption maxima (33), such that clinically measurable color vision deficits are observed using tests such as the FM-100 hue and Nagel anomaloscope.

Protanopia and deuteranopia represent two relatively common color anomalies. A third, tritanopia, represents a color vision disturbance in the blue-yellow axis; however, little is known of its clinical characteristics owing to its rarity in the general population. Subsequently, the study of tritanopic circadian physiology may prove to be difficult. This work would be important, as some investigators working with animal models have suggested that a photopigment with a peak absorbance similar to that of the short wavelength (blue) photoreceptor may be integral for circadian phototransduction (9, 14, 31, 32, 40, 41). Others have suggested that a rhodopsin-like photopigment, similar to that found in the rods of the retina, may mediate circadian photoreception (9, 14, 30, 42). Neither of these hypotheses has yet been carefully tested in the human model. Preliminary data of melatonin suppression using different wavelengths of light in healthy humans with normal color vision suggested a peak sensitivity for melatonin regulation at approximately 500 nm (11). The action spectrum for that pilot study, however, was not sufficient to clearly support any specific photoreceptor type as mediating the melatonin response to light.

Finally, although the identity of the photoreceptor(s) responsible for circadian phototransduction has yet to be characterized, work in blind humans and in retinally degenerate mice has suggested that a novel, as yet unidentified, system distinct from the visual system may be responsible (28, 31, 43, 44). Although not addressing the possibility of a novel photoreceptive system, the data presented here suggest that at least two of the four known photoreceptor cell types in the human retina may not be necessary for light-induced regulation of the melatonin secretory rhythm.

Our findings suggest that normal color perception is not necessary for light-mediated melatonin regulation in humans when using broad band white and green stimuli. Further, our data suggest that the long wavelength (red) and middle wavelength (green) color perceptual systems may themselves not be necessary for modulation of the melatonin secretory rhythm with a broad band stimulus. Knowledge of the photoreceptive system that mediates circadian regulation may have direct clinical applications in the development of light therapy modalities for shift workers, astronauts, and others with circadian dysregulation.

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