0021-972X/01/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 86, No. 1
Printed in U.S.A.

Human Melatonin Regulation is Not Mediated by the Three Cone Photopic Visual System

GEORGE C. BRAINARD*, JOHN P. HANIFIN, MARK D. ROLLAG, JEFFREY GREESON, BRENDA BYRNE, GENA GLICKMAN, EDWARD GERNER, BRITT SANFORD

Department of Neurology, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, and
Department of Anatomy, Uniformed Services University of Health Sciences, Bethesda, Maryland 20814.

ABSTRACT The aim of this study was to test if the three cone photopic visual system is the primary ocular photoreceptor input for human circadian regulation by determining the effects of different wavelengths on light-induced melatonin suppression. Healthy subjects with stable sleeping patterns (wake-up time 7:30 AM ± 12 min) and normal color vision were exposed at night to full-field 505 nm or 555 nm monochromatic stimuli or darkness for 90 min. Plasma collected before and after exposures was quantified for melatonin. Subjects exposed to 10 irradiances at 505 nm showed no significant differences across mean pre-exposure melatonin values (F=0.505). A sigmoidal fluence-response curve fitted to the melatonin suppression data (R^2=0.97) indicated that 9.34 x10^{12} photons/cm^2/sec induced a half-saturation response (ED_{50}) while 6.84 x10^{13} photons/cm^2/sec induced a saturation melatonin suppression response. Further, a dose of 4.19 x10^{13} photon/cm^2/sec at 505 nm was significantly stronger (P<0.01) than an equal photon dose at 555 nm for melatonin suppression. These data demonstrate that the cone system that mediates human photopic vision is not the primary photoreceptor system to transduce light stimuli for melatonin regulation.

Introduction

The underlying neuroanatomy and neurophysiology which mediate vision have been studied extensively over the past two centuries. More recently, the retinohypothalamic tract (RHT), a distinct neural pathway which supports circadian regulation by environmental light, has been shown to project from the retina to the suprachiasmatic nuclei (SCN) in the hypothalamus (1, 2). A well-defined multisynaptic neural pathway extends from the SCN to the pineal gland which transmits information about light and circadian time for entraining the rhythmic production and secretion of the hormone melatonin (1-4). In addition to synchronizing pineal indolamine circadian rhythms, ocular exposure to light during the night can acutely suppress melatonin synthesis and secretion (5, 6). Light-induced melatonin suppression is a well-defined, broadly used marker for photic input to the RHT and SCN (2, 4, 7, 8).

Currently, it is not known what photoreceptors transduce light stimuli for circadian regulation. Studies on animals with hereditary or light-induced retinal degeneration have raised the possibility that neither the rods nor the cones used for vision participate in light-induced melatonin suppression, circadian locomotor phase-shifts, or photoperiodic responses (8-12). Furthermore, bilateral removal of the eyes from rod-less, cone-less transgenic mice abolished light-induced circadian phase-shifting and acute melatonin suppression (8, 12). Recently, light-induced melatonin suppression and circadian entrainment have been demonstrated in humans with complete visual blindness (13) and with specific color vision deficiencies (14). The study on humans with color vision deficiencies showed that protanopic and deuteranopic subjects who lacked functioning long wavelength-sensitive cones (red, or L cones), and middle wavelength cone photoreceptors (green, or M cones), exhibited normal light-induced melatonin suppression and entrainment of the melatonin rhythm (14). Thus, by themselves, neither the red nor green cone system could be the primary input for melatonin regulation, at least in humans with color vision deficiencies. Together, the results from human and animal circadian studies on different forms of visual blindness suggest that melatonin regulation by light is controlled, at least in part, by photoreceptors which differ from the photoreceptors that mediate vision.

Recent studies with various vertebrate species have identified several new molecules which may serve as circadian photopigments. These putative photopigments include both opsin-based molecules, such as vertebrate ancient (VA) opsin and melanopsin, as well as non-opsin molecules like the cryptochromes (15-17). Among these new photopigments, only melanopsin has been specifically localized to the human retina (18). The molecular identification of these candidate photopigments and their localization in the retina and/or neural components of the circadian system make them well-suited to act as circadian phototransducers. Functional data confirming their direct role in circadian photoreception, however, are lacking.

The aim of this study was to test the hypothesis that the three cone system which supports photopic (daytime) vision is the primary input for pineal melatonin suppression in humans with normal, healthy eyes. The peak wavelength sensitivity of the photopic visual system is generally believed to be near 555 nm (19). If melatonin regulation is mediated primarily by the three cone photopic visual system, then 555 nm light would be the most potent wavelength for regulating melatonin secretion. Our data show that 505 nm is approximately four times stronger than 555 nm in suppressing melatonin. These results demonstrate that the ocular photoreceptor primarily responsible for pineal melatonin regulation in humans, is not the cone system that is believed to mediate photopic vision. This study is the first test of a specific photoreceptor system for melatonin regulation in humans with healthy, intact eyes.

*Address all correspondence to: Dr. G. C. Brainard, Department of Neurology, Jefferson Medical College, 1025 Walnut Street, Suite 310, Philadelphia, PA 19107

Subjects, Materials and Methods

Subjects. The healthy females (N=6) and males (N=10) in this study had a mean ± SEM age of 25.7 ± 0.8 yrs, demonstrated normal color vision as measured by the Ishihara and Farnsworth

Munsell D-100 tests (mean FM score: 64.2 ± 11.5), had a stable sleeping pattern (mean wake-up time 7:30 AM ± 12 min), and signed an approved IRB consent document before participating.

Light exposure protocol. Each experiment began at midnight when subjects entered a dimly lit room (10 lux). One drop of 0.5% Cyclopentolate HCl was placed in each eye to dilate the pupils, and blindfolds were placed over subjects' eyes. Subjects remained sitting upright in darkness for 120 min. While still blindfolded and just prior to 2:00 AM, a blood sample was taken by venipuncture. During light exposure, each subject's head rested in an ophthalmologic head holder facing a Ganzfeld apparatus that provided a concave, patternless, white reflecting surface encompassing the subject's entire visual field. The subjects were exposed to the light stimulus from 2:00 to 3:30 AM. During this 90 min exposure, subjects sat quietly, kept their eyes open and gazed at a fixed target dot in the center of the Ganzfeld dome. Subject compliance for keeping their eyes open and the subjects' pupil size were monitored by a miniature video camera. At 3:30 AM, a second blood sample was taken. Each subject was exposed to complete darkness from 2:00 to 3:30 AM on their control night and was tested with at least 6 days between each nighttime exposure. Plasma samples were assayed for melatonin by RIA (20). The minimum detection limit of the assay was 0.5 - 2.0 pg/mL. Control samples had 8% and 14% intra-assay coefficients of variation.

Light production and measurement. Experimental light stimuli were produced by xenon arc lamps (Photon Technology Int'l , Inc., Princeton, NJ) enclosed in a light-proof chamber and cooled by high-speed fans and water circulation. An exit beam of light from each source was directed by a parabolic reflector, and excess heat in this beam was reduced by a water filter. Monochromatic wavelengths (10-11 nm half-peak bandwidth) were produced by a grating monochromator and light irradiance was controlled by a manual diaphragm. The resulting light beam was directed into the top area of a Ganzfeld dome and reflected evenly off the walls into volunteers' eyes. The entire reflecting surface of the dome was coated with a white surface with a 95-99% reflectance efficiency over the 400 to 760 nm range. Routine measurement of the light irradiance ($\mu W/cm^2$) was done with a J16 Meter with a J6512 irradiance probe (Tektronix, Beaverton, OR). Experimental light stimuli reflected from the Ganzfeld domes were measured at volunteers' eye level immediately before and after the 90 min exposure. Additional measures were taken each half hour of the exposure to insure stimulus stability and enable intensity readjustment. Subjects in the 505 nm series were exposed to intensities ranging from 0.011 to 97 $\mu W/cm^2$ (a 3 log unit photon density range of 10^{10} to 10^{13} photons/cm^2). Subjects exposed to 555 nm received control or a 15 $\mu W/cm^2$ (4.2 x 10^{13} photons/cm^2) exposure.

Statistics. Two-tailed, Students' t tests were used to assess significance of raw melatonin change from 2:00 to 3:30 AM. These data were then converted to % control-adjusted melatonin change scores as described elsewhere (21). For the 505 nm data, sets of pre-exposure melatonin values and % control-adjusted melatonin change scores were analyzed with one-way, repeated measures ANOVA. Significant differences between groups were assessed with post-hoc Scheffe F-tests; alpha was set at 0.05. For the 505 nm mean % control-adjusted melatonin suppression data, the computer program Origin 6.0 (Microcal, Northampton, MA) was used to fit a fluence-response curve to a 4 parameter model as described elsewhere (22), and to test for goodness-of-fit of the data by coefficient of correlation.

Results

The full 505 nm data complement, from raw melatonin values to a final fluence-response curve, is illustrated in Figures 1-3. Across all nights of testing, there were no significant differences (F=0.85) between sets of pre-exposure melatonin values indicating that plasma levels were consistent across the different study nights. Figure 1 shows the mean ± SEM pre- and post-exposure melatonin values. One-way, repeated measures ANOVA showed a significant effect of light intensity on plasma melatonin % change scores (F=17.17, P<0.0001). Paired t tests demonstrated that compared to the 0 $\mu W/cm^2$ control night, all intensities at or above 5.5 $\mu W/cm^2$ significantly suppressed melatonin (P<0.05 or less). In contrast, irradiances of 2.8, 1.4 and 0.011 $\mu W/cm^2$ did not suppress melatonin compared to the control night (Scheffe F values: 0.97, 0.01 and 0.02, respectively). As illustrated in Figure 2, all melatonin data were converted to control-adjusted % change scores. As with the melatonin % change scores, ANOVA showed a significant effect of light intensity on plasma melatonin % control-adjusted change scores (F=13.59, P<0.0001).

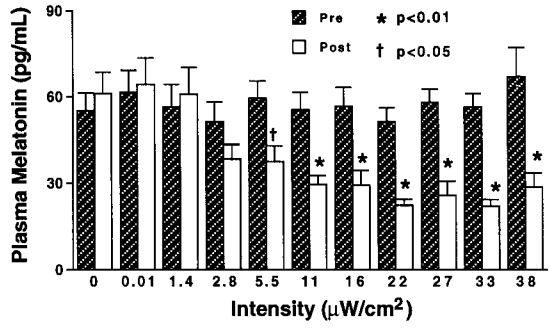

Figure 1: In this graph the bars represent group mean + SEM plasma melatonin values (N=8) before and after monochromatic light exposure at 505 nm. There were no significant variations across mean melatonin pre-light exposure values (F=0.85). Paired, two-tailed Students' t tests demonstrated which light intensities elicited a significant melatonin suppression.

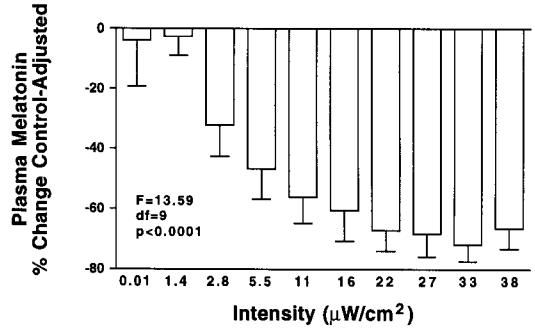

Figure 2: This graph illustrates group mean + SEM % control-adjusted melatonin change values (N=8) at 505 nm monochromatic light exposure. The figure shows that progressively higher light irradiance exposure produces increasingly greater melatonin suppression.

Figure 3 illustrates a best fit, sigmoidal fluence-response curve which plots melatonin % control-adjusted scores against stimulus photon density. The specific formula for this curve is included in the figure.

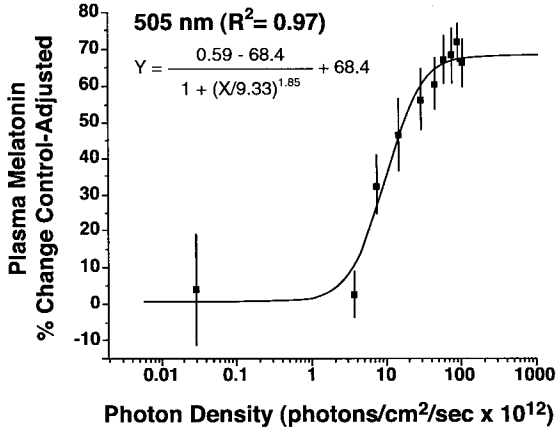

Figure 3: This figure demonstrates the fitted fluence-response curve for photon density and % control-adjusted melatonin suppression (N=8). Each data point represents one group mean ± SEM.

Subjects exposed to 555 nm received both control (0 $\mu W/cm^2$) and 15 $\mu W/cm^2$ (4.2 x 10^{13} photons/cm^2) exposures. For the control and light exposure nights, the mean pre-exposure raw melatonin scores were 64.4 ± 12.5 and 59.6 ± 6.2, while the mean post-exposure scores were 62.6 ± 10.5 and 49.1 ± 6.0, respectively. The modest drop in melatonin over the 90 min 555 nm light exposure period was not statistically significant (t=1.69, df=7, P=0.14). For comparison of responses to 505 nm and 555 nm, Figure 4 illustrates % control-adjusted melatonin suppression relative at equal photon densities across the two wavelengths. These data reveal that 505 nm is significantly stronger than 555 nm in suppressing melatonin (t=-3.04, df=14, P<0.01).

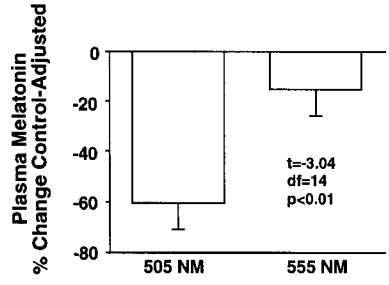

Figure 4: The bars represent group mean + SEM values relative to an equal photon dose of 4.2 x 10^{13} photons/cm^2. These data show that the 505 nm % control-adjusted plasma melatonin suppression is significantly stronger than that for 555 nm.

Discussion

The data presented here demonstrate that: 1) there is a clear fluence-response relationship between graded light intensities of 505 nm light and melatonin suppression, and 2) that 505 nm light is significantly stronger than 555 nm light for suppressing melatonin in healthy, human subjects. Previous studies with animals and humans have illustrated fluence-response relationships for melatonin suppression and circadian phase-shifting with exposure to monochromatic light (23-25) as well as white light (22, 26). A recent study on human subjects suggests that a four parameter curve is optimum for modeling light-induced melatonin suppression and circadian phase shifting (27). That contention matches our own earlier animal data (22) as well as the 505 nm data reported here.

The demonstration that 505 nm light is more potent than 555 nm light for controlling melatonin has important basic science and clinical implications. In humans, it is well-established that higher levels of ocular illumination are required for stimulating the circadian system than for supporting vision (6, 25, 30). Consequently, many investigators have considered the three cone photopic visual system to be responsible for stimulating circadian and neuroendocrine responses since this part of the visual system is responsive to "bright" daytime levels of illumination. Over the past 20 years most of the published literature on human circadian responses to light reports light levels in terms of illuminance (lux, lumens) which is a specific measure based on the traditional sensitivity curve of the photopic visual system. The peak wavelength sensitivity of that curve is 555 nm (19). Indeed, some researchers have argued that their data support the notion that the visual cones are involved in circadian phase-shifting in humans (28). If melatonin regulation is mediated primarily by the three cone photopic visual system, then 555 nm light should be the most potent wavelength for regulating melatonin. Our data do not support this hypothesis. On the contrary, 505 nm is significantly stronger, photon for photon, than 555 nm in suppressing melatonin. The clinical implication of this result is that it is not optimum to use photometric values (lux) for quantifying light used therapeutically in patients with certain sleep disorders or circadian disruption due to shiftwork or intercontinental jet travel as is the current standard practice (29). It should be noted, however, that the data presented here involve only the melatonin suppression response in healthy humans. It remains to be determined if there are similar wavelength sensitivities for circadian phase-shifting or light therapy for clinical disorders.

Ultimately, these studies open the door for redefining how light should be measured relative to the circadian system. The best circadian measurement system would match the action spectrum for human circadian regulation. That action spectrum would not only elucidate the relative circadian potencies of different wavelengths, but it should help identify the photoreceptor that initiates signals from the retina to the SCN. Unfortunately, an action spectrum has yet to be established for any circadian response in humans. It remains possible that one of the "classic" visual photopigments such as rhodopsin or the short wavelength sensitive (blue) cone opsin mediates circadian

phototransduction. Alternatively, one of the new opsin or non-opsin molecules that have been proposed as circadian photopigments (15-17) may prove to serve this function. Until the action spectrum for circadian regulation in humans has been clarified, it remains reasonable to continue using photometric terminology and measurements for characterizing light that regulates the circadian system.

In summary, monochromatic 505 nm light suppressed melatonin in a fluence-response manner, and is approximately four times stronger than a 555 nm stimulus at an equal photon dose for melatonin suppression. These data demonstrate that the three cone system that is believed to mediate human photopic vision is not the primary photoreceptor system to transduce light stimuli for melatonin regulation.

Acknowledgments

The authors gratefully acknowledge the technical advice and assistance of William Thornton, PhD, William Coyle, James Gardner, Robert Glasgow, Christine Alocillo, Donna Wittkowski, Jeff Santman and Jon Cooke. Their input was invaluable to this project. The inspiration for this work came from the 281 series of the Edgar Cayce readings. This work was supported by grants from NIH RO1NS36590 (to GCB) and NSF IBN9809916 (to MDR).

References

1. **Moore RY, Lenn NJ** 1972 A retinohypothalamic projection in the rat. J Comp Neurol. 146:1-14.
2. **Klein DC, Moore RY, Reppert SM, eds.** 1991 Suprachiasmatic Nucleus: The Mind's Clock. Oxford: Oxford University Press; 5-456.
3. **Schwartz WJ, Busis NA, Hedley-Whyte ET** 1986 A discrete lesion of ventral hypothalamus and optic chiasm that disturbed the daily temperature rhythm. J Neurol. 233:1-4.
4. **Arendt J** 1998 Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology. Rev. Reprod. 3, 13-22.
5. **Klein DC, Weller JL** 1972 Rapid light-induced decrease in pineal serotonin N-acetyltransferase activity. Science. 177:532-533.
6. **Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP** 1980 Light suppresses melatonin secretion in humans. Science. 210:1267-1269.
7. **Brainard GC, Rollag MD, Hanifin JP** 1997 Photic regulation of melatonin in humans: ocular and neural signal transduction. J Biol Rhythms. 12:537-546.
8. **Lucas RJ, Foster RG** 1999 Neither functional rod photoreceptors nor rod or cone outer segments are required for the photic inhibition of pineal melatonin. Endocrinology. 140:1520-1524.
9. **Webb SM, Champney TH, Lewinski AK, Reiter RJ** 1985 Photoreceptor damage and eye pigmentation: influence on the sensitivity of rat pineal N-acetyltransferase activity and melatonin levels to light at night. Neuroendocrinology. 40:205-209.
10. **Goto M, Ebihara S** 1990 The influence of different light intensities on pineal melatonin content in the retinal degenerate C3H mouse and the normal CBA mouse. Neurosci Lett. 108:267-272.
11. **Foster RG, Provencio I, Hudson D, Fiske S, DeGrip W, Menaker M** 1991 Circadian photoreception in the retinally degenerate mouse (rd/rd). J Comp Physiol [A]. 169:39-50.
12. **Freedman MS, Lucas RJ, Soni B, et al.** 1999 Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science. 284:502-504.
13. **Czeisler CA, Shanahan TL, Klerman EB, et al.** 1995 Suppression of melatonin secretion in some blind patients by exposure to bright light. N Engl J Med. 332:6-11.
14. **Ruberg FL, Skene DJ, Hanifin JP, et al.** 1996 Melatonin regulation in humans with color vision deficiencies. J Clin Endocrinol Metab. 81:2980-2985.
15. **Soni BG, Foster RG** 1997 A novel and ancient vertebrate opsin. FEBS Lett. 406:279-283.
16. **Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD** 1998 Melanopsin: an opsin in melanophores, brain, and eye. Proc Natl Acad Sci U S A. 95:340-345.
17. **Miyamoto Y, Sancar A** 1998 Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. Proc Natl Acad Sci U S A. 95:6097-6102.
18. **Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD** 2000 A novel human opsin in the inner retina. J Neurosci. 20:600-605.
19. **Rodieck RW** 1998 The First Steps in Seeing. Sunderland, MA: Sinauer Associates, Inc.; 1-562.
20. **Rollag MD, Niswender GD** 1976 Radioimmunoassay of serum concentrations of melatonin in sheep exposed to different lighting regimens. Endocrinology. 98:482-489.
21. **Gaddy JR, Rollag MD, Brainard GC** 1993 Pupil size regulation of threshold of light-induced melatonin suppression. J Clin Endocrinol Metab. 77:1398-1401.
22. **Brainard GC, Richardson BA, King TS, Matthews SA, Reiter RJ** 1983 The suppression of pineal melatonin content and N-acetyltransferase activity by different light irradiances in the Syrian hamster: a dose-response relationship. Endocrinology. 113:293-296.
23. **Podolin PC, Rollag MD, Brainard GC** 1987 The suppression of nocturnal pineal melatonin in the Syrian hamster: dose-response curves at 500 nm and 360 nm. Endocrinology. 121:266-270.
24. **Brainard GC, Lewy AJ, Menaker M, et al.** 1988 Dose-response relationship between light irradiance and the suppression of melatonin in human volunteers. Brain Res. 454:212-218.
25. **Nelson DE, Takahashi JS** 1991 Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. Brain Res. 554:272-277.
26. **Boivin DB, JF Duffy, RE Kronauer, CA Czeisler** 1996 Dose-response relationships for resetting of human circadian clock by light. Nature 379, 540-542.
27. **Zeitzer JM, Dijk D-J, Kronauer RE, Brown EN, Czeisler CA** 2000 Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol. in press.
28. **Zeitzer JM, Kronauer RE, Czeisler CA** 1997 Photopic transduction implicated in human circadian entrainment. Neurosci Lett. 232:135-138.
29. **1995 Special Issue: Task force report on light treatment for sleep disorder** J. Biol. Rhythms 10, 99-176.
30. **Czeisler CA, Allan JS, Strogatz SH, Ronda JM, Sanchez R, Rios CD, Freitag WO, Richardson GS, Kronauer RE** 1986 Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. Science 233, 667-671.