Inferior Retinal Light Exposure Is More Effective than Superior Retinal Exposure in Suppressing Melatonin in Humans

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Abstract Illumination of different areas of the human retina elicits differences in acute light-induced suppression of melatonin. The aim of this study was to compare changes in plasma melatonin levels when light exposures of equal illuminance and equal photon dose were administered to superior, inferior, and full retinal fields. Nine healthy subjects participated in the study. Plexiglass eye shields were modified to permit selective exposure of the superior and inferior halves of the retinas of each subject. The Humphrey Visual Field Analyzer was used both to confirm intact full visual fields and to quantify exposure of upper and lower visual fields. On study nights, eyes were dilated, and subjects were exposed to patternless white light for 90 min between 0200 and 0330 under five conditions: (1) full retinal exposure at 200 lux, (2) full retinal exposure at 100 lux, (3) inferior retinal exposure at 200 lux, (4) superior retinal exposure at 200 lux, and (5) a dark-exposed control. Plasma melatonin levels were determined by radioimmunoassay. ANOVA demonstrated a significant effect of exposure condition ($F = 5.91, p < 0.005$). Post hoc Fisher PLSD tests showed significant ($p < 0.05$) melatonin suppression of both full retinal exposures as well as the inferior retinal exposure; however, superior retinal exposure was significantly less effective in suppressing melatonin. Furthermore, suppression with superior retinal exposure was not significantly different from that of the dark control condition. The results indicate that the inferior retina contributes more to the light-induced suppression of melatonin than the superior retina at the photon dosages tested in this study. Findings suggest a greater sensitivity or denser distribution of photoreceptors in the inferior retina are involved in light detection for the retinohypothalamic tract of humans.

Key words circadian rhythm, visual fields, retina, melatonin, photoreceptor, light

It has been well established that the retinohypothalamic tract (RHT) is responsible for photic entrainment of the circadian system (Klein et al., 1991). Photic information, relayed from the retina to the suprachiasmatic nuclei (SCN) in the hypothalamus, is subsequently transmitted via a multisynaptic pathway to the

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pineal gland, which regulates melatonin synthesis and secretion (Klein et al., 1991). In addition to synchronizing the circadian melatonin rhythm, nighttime light exposure of the eye(s) can acutely inhibit the pineal enzyme serotonin-N acetyltransferase and therefore suppress circulating melatonin levels. This acute light-induced suppression of melatonin has been useful in studying ocular, neural, and molecular elements of circadian physiology (Klein and Weller, 1972; Klein et al., 1991; Brainard et al., 1997; Arendt, 1998).

Studies have indicated an anatomical and functional dichotomy between the visual and circadian pathways at the photoreceptor, ganglion cell, and neural projection levels in humans and other mammalian species (Schwartz et al., 1986; Magnin et al., 1989; Card, 1994; Moore et al., 1995; Provencio and Foster, 1995; Brainard et al., 2001a, 2001b; Thapan et al., 2001). Conservation of circadian responses and maintenance of cone sensitivity threshold and range in rodless mice once seemed to implicate the involvement of a cone-like photoreceptor (Nelson and Takahashi, 1991; Provencio and Foster, 1995; Lupi et al., 1999). However, recent evidence from rodless-coneless transgenic mice (Freedman et al., 1999; Lucas et al., 1999), melatonin suppression by light in visually blind humans, and humans with color vision deficiencies (Czeisler et al., 1995; Ruberg et al., 1996) suggests that a novel photoreceptor may be primarily involved in circadian regulation. The recent action spectra that have characterized the peak wavelength range for human melatonin suppression to be 446 to 477 nm suggest circadian photoreception employs a photoreceptor system distinct from the classic human photoreceptors for vision (Brainard et al., 2001a, 2001b; Thapan et al., 2001).

A variety of photopigments could be implicated in circadian phototransduction, including vertebrate ancient opsin (Soni and Foster, 1997) encephalopsin (Blackshaw and Snyder, 1999), and cryptochrome (Miyamoto and Sancar, 1998). Melanopsin, a strong candidate circadian photopigment, has been localized in both the rodent and human neural retina (Provencio et al., 1998, 2000). In rodents, melanopsin has been found both in a specific subtype of retinal ganglion cells (RGCs) that project to the SCN (Gooley et al., 2001) and in an expansive ganglion cell dendritic arbor that may form a "photoreceptive net" for circadian phototransduction (Provencio et al., 2002). Moreover, a study on rats has shown that the ganglion cells with projections to the SCN are intrinsically responsive to light. The light responses appear to parallel those of photic entrainment and melatonin suppression, suggesting that these ganglion cells may be the primary photoreceptors involved in circadian regulation (Berson et al., 2002). In addition, these same intrinsically photosensitive retinal ganglion cells were found to be melanopsin positive whereas other ganglion cells were melanopsin negative (Gooley et al., 2001). Two studies, in fact, have demonstrated an uneven distribution of these melanopsin-positive ganglion cells in the retinas of rats (Hannibal et al., 2002; Hattar et al., 2002).

There is additional evidence suggesting that all regions of the retina are not equally involved in the process of circadian photoreception. Animal studies, for example, have shown diverse variations in retinal photoreceptor and ganglion cell topography (Cooper et al., 1993; Szel et al., 1996, 2000). In addition, varying the retinal surface area illumination in humans has demonstrated differences in acute melatonin suppression (Adler et al., 1992; Gaddy et al., 1992; Brainard et al., 1997; Wang et al., 1997; Visser et al., 1999; Lasko et al., 1999). Such studies have suggested a nonhomogenous distribution of the circadian photoreceptors, variations in sensitivity, and/or the possible spatial summation of photic stimuli across the human retinas.

Studies testing specific regional exposures to light have included comparisons of central/peripheral, nasal/temporal, and superior/inferior retinal exposures. A study by Gaddy et al. (1992), using roughly selective exposure fields, found that generally higher levels of illuminance were required to produce significant melatonin suppression with inferior retinal illumination as compared to full retinal exposure. It was also found that light exposure to both retinas resulted in a greater suppression of melatonin than exposures of the same illuminance and equal photon dose to only one retina (Brainard et al., 1997; Wang et al., 1997). Adler et al. (1992) found that melatonin suppression in humans was equivalent with central and peripheral illumination, while Visser et al. (1999) later found that illumination of the nasal portion of the retina caused a significantly greater suppression in melatonin than temporal region exposures. There have been conflicting results when observing superior versus inferior retinal exposures. One study showed that inferior retinal exposures suppressed melatonin more than superior retinal exposures did, and another study demonstrated no significant difference between these two retinal regions (Lasko et al., 1999; Visser et al., 1999). Although there was some disparity in the results of these earlier studies and the methods employed used
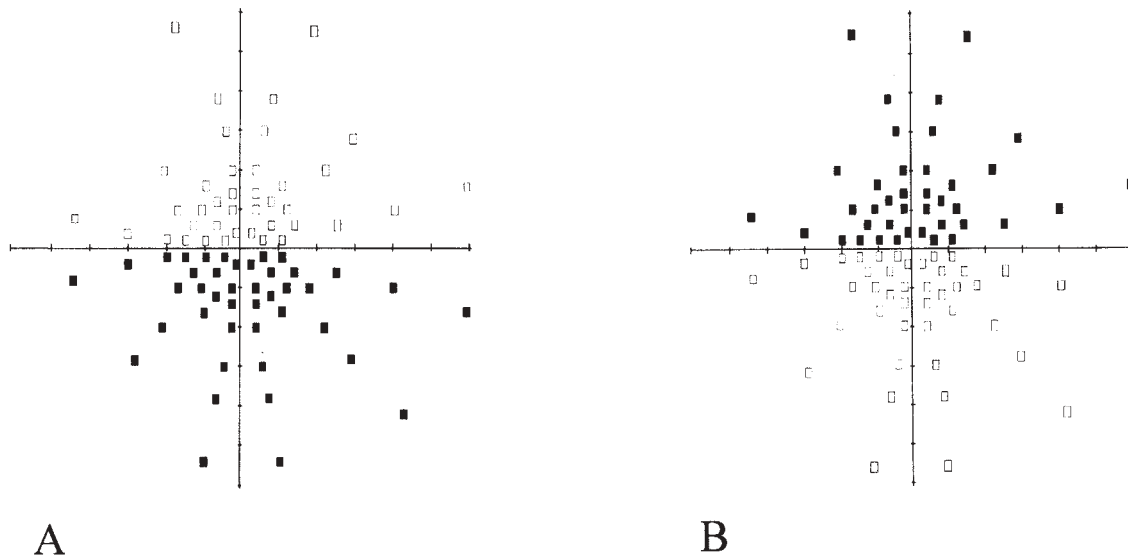
Figure 1. (A) Results of a full-field, 81-point single intensity screening test for the right eye of a subject wearing eyeshields modified for the inferior retinal exposure condition. **(B)** Results of a full-field, 81-point single intensity screening test for the right eye of a subject wearing eyeshields for superior retinal exposure. The solid squares in bold indicate those not seen by the subject. The open squares indicate points that were seen by the subject. All points have been enlarged for clarity.

somewhat crude estimations of retinal exposure regions, the body of research identified a clear need to further investigate the variability of retinal exposure region in the light-induced suppression of melatonin in humans.

This study compared the change in plasma melatonin with light exposures of superior, inferior, and full retinal regions of equal illuminance and equal photon dose in the same human subjects. As light entering the human eye creates an inverted image on the retina, upper visual field determination was used to precisely identify the inferior retinal exposure region, and conversely, lower visual field measurement was treated as the equivalent to the superior retinal region. Based on the aforementioned studies, it was hypothesized that light exposure to the inferior portion of the retina would exhibit greater plasma melatonin suppression than superior region exposures and that full retinal exposures would cause a stronger response than both half-exposure conditions.

METHODS

The subjects consisted of 5 females and 4 males in good health (mean age ± SEM = 24.9 ± 0.9). All subjects reported regular sleep patterns and signed institutional review board–approved consent forms before participating. Three of the 5 female subjects were on oral contraceptives. The study was not confined to a particular time of year, and therefore seasonal differences in melatonin values between subjects were not examined. Prior to subject participation in study nights, the Humphrey Visual Field Analyzer II, full-field, 81-point screening test was used both to confirm the absence of any visual field deficits and to delineate between the upper and lower retinal fields when designing individually modified plexiglass eyeshields for each subject. During the screening test, subjects were required to fixate their gaze on a central marker in the dome. Although fixation losses were electronically monitored during full-field exposures, the Humphrey Visual Field Analyzer could not track subjects' eyes during half-field exposures as a full view of the eye was obstructed by the modified half retinal exposure condition plexiglass shields. Therefore, a monitor display of subjects' eyes was observed visually by staff administering the screening test, and subjects were verbally reminded to keep gaze fixated on the central marker. The full-field, 81-point screening test field is divided into quadrants by an x- and y-axis, with 42 of the 81 points presented to the bottom visual field (below the x-axis) and 39 presented to the top visual field (above the x-axis) (see Fig. 1).

Half retinal exposure scores for each subject while wearing modified eyeshields were required to have ≥ 90% similarity to the ideal score for each condition, and plexiglass shields were adjusted until these stan-

dards were met for each exposure condition in each subject. For example, an ideal score for an inferior retinal exposure condition (upper visual field exposure) would indicate all points above the *x*-axis were seen and none below the *x*-axis were seen by subjects. Individualized superior and inferior retinal exposure shields were constructed by partially masking plexiglass safety glasses (Uvex, Smithfield, Rhode Island, USA) using opaque black electrical tape to obstruct light from entering the opposing retinal field. While adapted safety glasses were used for superior and inferior retinal exposure conditions, unmodified glasses were worn by subjects on nights of full retinal exposure to account for any changes in transmittance due to the translucent unblocked portion of the modified glasses' lenses.

For all exposure conditions, polychromatic white light from a 150-watt halogen lamp was transmitted through fiber optic cables to the homogenous reflecting surface of the upper aspect of a Goldman perimeter. Spectral irradiance measurements of each Ganzfeld dome were made with an Ocean Optics Spectrometer (Model S2000) calibrated to a 200-watt NIST Traceable Standard of Spectral Irradiance (Model OL 220M, serial number M1004a) by Solar Light Company (Philadelphia, PA, USA). Based on the resultant spectral power distributions between 400 and 800 nm, a calculated conversion was made from measured lux to photon flux. For the full retinal field exposure at 100 lux, there was an approximate average retinal photon flux of 1.5 micromoles/m^2/sec or 9.2×10^{13} photons/cm^2/sec. Masking of the superior and inferior retinal fields reduces the total photon dose to the retina. Our best prediction is that this reduction is approximately 50% of the full-field exposure since visual perception is reduced by 50% as documented by the Humphrey Visual Field Analyzer described above. Hence, the illuminances of partial retinal field exposures were doubled to 200 lux to yield approximately equal photon doses. Before, during, and after light exposures, illuminance was measured with a Minolta Chroma Meter (Minolta Camera Co., Osaka, Japan) to prepare the appropriate experimental condition, ensure the maintenance of determined illuminance, and enable any necessary adjustments. In addition to the routine measurement of illuminance, pupil dilation and gaze behavior were also monitored to ensure constancy of exposure for all conditions. Subjects' heads were kept in a constant position by an ophthalmological head holder. In addition, subjects were instructed to fixate their gaze on a central location in the dome, similarly to that of the Humphrey Visual Field Analyzer II screening portion of the protocol. The central marker is the end of a telescoping lens that lays flat against the back of the Ganzfeld dome. The lens allowed for the visual monitoring and establishment of constant position of glasses, direction of gaze, and pupil dilation routinely during light exposure.

Each subject completed 5 nights, with at least 1 week between each test night. Subjects arrived at 11:45 PM. Eyes were dilated with 1 to 2 drops of 0.5% cyclopentolate HCl and were blindfolded for 2 hours. At 2:00 AM, blood samples were taken from each subject. Blindfolds were then removed, glasses for appropriate exposure condition were applied, and light exposure began. Subjects received 90-min exposures for each of the five randomly sequenced test conditions: (1) full retinal exposure at 200 lux, (2) full retinal exposure at 100 lux, (3) superior retinal exposure at 200 lux, (4) inferior retinal exposure at 200 lux, and (5) dark control condition. Postexposure blood samples were obtained at 3:30 AM, and all plasma melatonin levels were determined by a modification of the radioimmunoassay developed by Rollag and Niswender (1976). While pre– and post–plasma melatonin were always assayed together for each subject in each condition, all samples were not assayed together. The intraassay coefficients of variation averaged 16% and 8%, respectively. Intraassay coefficient of variation was calculated from four replicates of standards from a large pool dispersed throughout the assay. The mean and standard deviation were obtained, and the intraassay percentage coefficient of variation was determined as (SD/mean) $\times$ 100. The mean was recorded for each of the four replicates for each assay using samples obtained from the same original pool. After all assays were done, the interassay coefficient of variation from these means was determined. The interassay coefficients of variation in control samples with 18 pg/ml and 83 pg/ml immunoreactivity were 9.8% and 6.9%, respectively.

A two-way, repeated measures ANOVA was employed to assess differences in raw melatonin levels over time and between conditions. Two-tailed, paired *t* tests were used to determine changes in raw melatonin for preexposure and postexposure values. Consistency of 2:00 AM preexposure melatonin levels between study nights was analyzed by one-way ANOVA with significance set at the 95% confidence level. The change in melatonin for each test condition was expressed as a percentage change from the
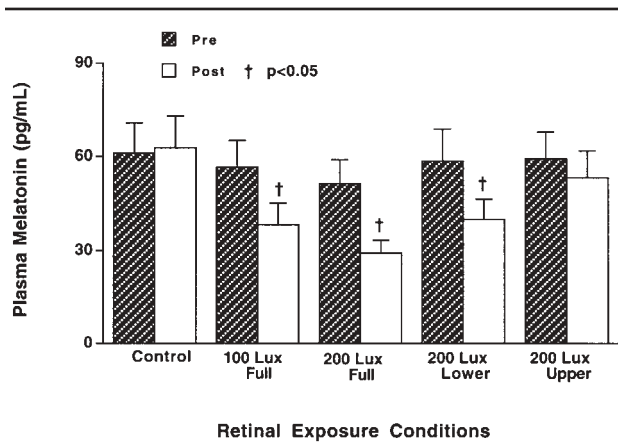
Figure 2. Two-way ANOVA shows that there is a significant interaction between time and condition on raw melatonin values ($p < 0.04$). In addition, one-way ANOVA shows that all preexposure means are not significantly different from each other ($p = 0.15$). Full retinal exposures at 100 and 200 lux and inferior retinal exposure at 200 lux significantly suppress plasma melatonin levels. In contrast, the superior retinal exposure at 200 lux and the dark control condition do not show a significant difference for preexposure versus postexposure values. Bars indicate mean + SEM plasma melatonin.
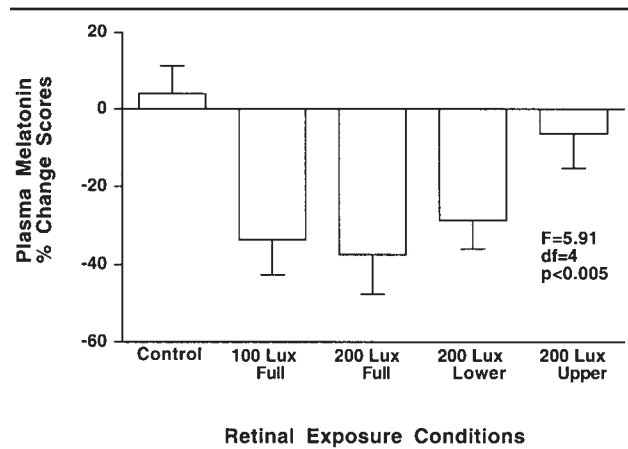

Figure 3. Percentage changes in melatonin for all experimental nights (mean + SEM). Both full retinal and inferior retinal exposures were statistically different from both superior retinal and dark control conditions ($p < 0.005$). Furthermore, percentage change scores of full retinal exposures at 100 and 200 lux and inferior retinal exposures were not statistically different from one another.

preexposure melatonin level, with a positive percentage change indicating an increase and a negative percentage change demonstrating a decrease in melatonin levels. Percentage change values were then analyzed by ANOVA. Post hoc comparisons between individual groups were done with the Fisher PLSD.

RESULTS

All subjects entered into the study were determined to have intact visual fields as determined by each subject's score of ≥ 79 points out of 81 points seen for full-field exposures. The mean scores for the Humphrey Visual Field Analyzer II full-field, 81-point screening tests for subjects wearing modified eyeshields for partial retinal exposure conditions had a similarity of ≥ 90% to the ideal score for each condition (see Fig. 1). These results demonstrate that the plexiglass eyeshields limited exposure to the appropriate retinal hemisphere.

Two-way, repeated measures ANOVA demonstrated a significant effect of time ($F[1] = -22.81$, $p < 0.0001$) and an interaction of time and exposure condition ($F[4] = 2.95$, $p < 0.04$). Consistency of baseline plasma melatonin levels across all study nights was demonstrated as preexposure melatonin levels were found to be statistically equivalent across all test conditions by ANOVA ($F = 1.8$, $p = 0.15$). As illustrated in

Fig. 2, full retinal exposures of both 100 lux and 200 lux as well as inferior retinal exposure of 200 lux showed significant suppression of plasma melatonin levels ($p < 0.05$). In contrast, superior retinal exposure of 200 lux and dark control conditions do not show a significant difference for preexposure versus postexposure values.

Fig. 3 displays melatonin data that have been converted to percentage change scores to reduce intersubject variance. Melatonin suppression was significantly different across five conditions ($F = 5.91$, $p < 0.003$). Post hoc comparisons showed that the superior retinal exposure was not statistically different from the control exposure ($p = 0.33$). Full retinal exposures at 100 lux and 200 lux as well as the inferior retinal exposure were statistically equivalent to each other ($p = 0.40$) yet significantly different from superior retinal and dark control conditions ($F = 21.58$, $p < 0.05$). Furthermore, the inferior retinal exposure of 200 lux was statistically similar to both full retinal exposures of equal illuminance ($p = 0.41$) and equal photon dosage ($p = 0.64$).

DISCUSSION

The data clearly demonstrate stronger melatonin suppression with inferior retinal exposures versus superior retinal exposures. In addition, full retinal exposure of equal illuminance (200 lux) as well as full

retinal exposure of equal photon dose (9.2×10^{13} photons/cm^2/sec) were equivalent in potency to the inferior retinal exposure condition. Surprisingly, exposure of the superior retina did not elicit a melatonin suppression that was significantly different from the dark control condition. Hence, there appears to be regional specialization in the retina for photoreception that mediates light-induced suppression of melatonin in humans.

This result differs from a previous study that showed higher levels of illuminance were required to elicit melatonin suppression when the inferior retina was exposed as compared to full-field exposures (Gaddy et al., 1992). That trial, however, differed significantly in study design compared to the experiment reported here. Specifically, the earlier study (1) tested two different light sources with substantively different color temperatures (5200 and 2800 °K) at much higher light illuminances, (2) did not employ rigorously controlled or characterized partial retinal light exposures, and (3) did not control or measure pupil size. It is now known that both pupil dilation and light spectrum play a crucial role in the melatonin suppression response in humans (Gaddy et al., 1993; Brainard et al., 2001a). Two other studies testing the role of retinal field variations in melatonin suppression may have yielded conflicting results due to similar limitations, including rudimentary identification of exposure region and lack of pupillary controls. One study found a significantly stronger melatonin suppression with nasal versus lateral retinal exposures but no differences in superior versus inferior exposures (Visser et al., 1999). Another study, however, demonstrated significantly stronger melatonin suppression with inferior as compared to superior retinal exposure (Lasko et al., 1999).

The study reported here is the first human melatonin experiment to employ the precise quantification of visual fields and the consequent determination of the retinal exposure region for each subject by using the Humphrey Visual Field Analyzer. Each subject's data showed no significant deviation from the x-axis division for both half-exposure conditions (see Fig. 1). Although the eyeshields blocked a large portion of scattered photons from entering regions opposite to selected exposure conditions, a modicum of intraocular light scatter could be anticipated even with relatively low illuminances of 100 and 200 lux of light. Since the superior retinal exposures of 200 lux were not significantly different from the dark control

condition, it appears that whatever intraocular light scatter may have occurred did not elicit a significant physiological response. It is possible, however, that the light levels employed were not sufficiently sensitive to detect a difference in response associated with a twofold change in photon dose as the light-induced melatonin suppression with full retinal exposures of 200 and 100 lux were not significantly different.

Previous studies have suggested that the circadian system may function as a "photon counter," meaning that the number of photons in a light exposure over extended periods of time or across the photoreceptive retinal surface are simply added together (Takahashi et al., 1984; Nelson and Takahashi, 1991; 1999; Foster et al., 1993). In the data reported here, partial retinal exposures of equal photon dose to full retinal exposures failed to produce similar results when the light stimulus was isolated to the superior retina. This suggests that integration of photon capture does not occur uniformly across the entire retina. In congruence with these findings, an earlier study found that light exposure to both eyes caused a greater suppression of melatonin than did exposures of equal photon dose to only one eye (Wang et al., 1997).

Among the various retinal photopigments that have been implicated in circadian phototransduction, melanopsin has been localized in both the rodent and human (Provencio et al., 1998, 2000). Although the relative distribution of retinal melanopsin-positive cells in humans has yet to be determined, two studies have demonstrated a higher density of melanopsin-positive cells in the superior and temporal regions of the retinas of rats (Hannibal et al., 2002; Hattar et al., 2002). If the primate distribution of SCN projecting cells matches that of rodents, it would suggest that it may not be the morphological distribution of RGCs in the retina, but rather a difference in sensitivity, accounting for physiological differences between retinal regions in melatonin suppression in humans.

Although several lines of evidence indicate that a photopigment different from the classical visual photopigments provides the primary input to the circadian system (Czeisler et al., 1995; Ruberg et al., 1996; Freedman et al., 1999; Lucas et al., 1999; Brainard et al., 2001a, 2001b; Thapan et al., 2001), recent studies suggest there are inputs from the visual rods and cones to neurons in the SCN (Aggelopoulos and Meissl, 2000) as well as the olivary pretectal nucleus (Lucas et al., 2001). A study of humans with color vision deficits showed that protanopic and deuteranopic observers

who lack functioning long-wavelength-sensitive cones (L-cones) and middle-wavelength-sensitive cones (M-cones) exhibited normal light-induced melatonin suppression and entrainment of the melatonin rhythm (Ruberg et al., 1996). However, due to the rarity of tritanopia, the potential contribution of S-cones in human circadian transduction has not yet been thoroughly examined. Given the increased responsiveness of the inferior retina in terms of melatonin suppression, it may be useful to examine potential S-cone involvement in circadian transduction as a higher density of S-cones have been found in the inferior region of the retina of some primates (Dkhissi-Benyaha et al., 2001). Most previous studies examining cone distribution in humans employed inferential methods based on similarity of cell distribution to that suggested by psychophysics; however, a later study using a more direct approach did not find a topographic separation of the different cone types in humans (Curcio et al., 1991).

Although probing differences in regional retinal sensitivity for retinohypothalamic input cannot provide the identity of circadian photoreceptors, discerning variations in the contributions of different retinal areas may allow for comparisons with morphological studies of various retinal photopigments as well as open the door to a variety of practical applications. Light treatment has been used clinically in the treatment of individuals with winter depression, selected sleep disorders, and circadian disruption (Lam, 1998). If the input system for light-induced melatonin suppression is the same input system for the therapeutic responses to light treatment, it will be important to fully characterize regional differences of retinal sensitivity and optimize light source geometry to elicit maximum therapeutic benefits. In this study, exposure of the entire retina was not necessary to produce suppression of melatonin. Not only did partial exposure to the inferior retinas produce significant melatonin suppression but there was also no statistical difference between suppressive qualities with inferior retinal exposure as compared to the full retinal exposures of equal illuminance and equal photon dosage. This suggests that light treatments that direct light primarily to the inferior retina may be adequate to produce therapeutic benefits in affective and circadian disorders. Conversely, directing light primarily to the superior retina may allow people to have visual stimulation while minimizing the effects of light on melatonin and circadian regulation.

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