Dim Light Adaptation Attenuates Acute Melatonin Suppression in Humans

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Abstract Studies in rodents with retinal degeneration indicated that neither the rod nor the cone photoreceptors obligatorily participate in circadian responses to light, including melatonin suppression and photoperiodic response. Yet there is a residual phase-shifting response in melanopsin knockout mice, which suggests an alternate or redundant means for light input to the SCN of the hypothalamus. The findings of Aggelopoulos and Meissl suggest a complex, dynamic interrelationship between the classic visual photoreceptors and SCN cell sensitivity to light stimuli, relative to various adaptive lighting conditions. These studies raised the possibility that the phototransductive physiology of the retinohypothalamic tract in humans might be modulated by the visual rod and cone photoreceptors. The aim of the following two-part study was to test the hypothesis that dim light adaptation will dampen the subsequent suppression of melatonin by monochromatic light in healthy human subjects. Each experiment included 5 female and 3 male human subjects between the ages of 18 and 30 years, with normal color vision. Dim white light and darkness adaptation exposures occurred between midnight and 0200 h, and a full-field 460-nm light exposure subsequently occurred between 0200 and 0330-h for each adaptation condition, at 2 different intensities. Plasma samples were drawn following the 2-h adaptation, as well as after the 460-nm monochromatic light exposure, and melatonin was measured by radioimmunoassay. Comparison of melatonin suppression responses to monochromatic light in both studies revealed a loss of significant suppression after dim white light adaptation compared with dark adaptation ($p < 0.04$ and $p < 0.01$). These findings indicate that the activity of the novel circadian photoreceptive system in humans is subject to subthreshold modulation of its sensitivity to subsequent monochromatic light exposure, varying with the conditions of light adaptation prior to exposure.

Key words adaptation, circadian rhythm, melanopsin, melatonin, pineal, photoreception, suprachiasmatic nucleus

Recent studies have begun to characterize the novel photoreceptive system that provides input to the human retinohypothalamic tract. Elucidation of this physiology has the potential to benefit the health, productivity, and well-being of humans in venues spanning from everyday life to space travel (Lam, 1998; Dijk et al., 2001). The acute suppression of plasma melatonin by light is a well-defined means

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JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 21 No. 5, October 2006 394-404
DOI: 10.1177/0748730406292391

of measuring the action of this novel, nonvisual photoreceptive system (Brainard et al., 1997). Recently, action spectra developed in healthy human subjects have shown that monochromatic light in the range of 446 to 477 nm is the peak wavelength range for melatonin suppression in humans (Brainard et al., 2001a; Thapan et al., 2001). Another study has shown that 460-nm monochromatic light is significantly stronger than 555 nm for phase-shifting the circadian pacemaker of healthy humans (Lockley et al., 2003). Together, these findings indicate that the primary circadian photoreceptor system has a peak wavelength sensitivity distinct from that of the classic visual photoreceptors.

Studies in rodents with retinal degeneration indicated that neither the rod nor the cone photoreceptors obligatorily participate in circadian responses to light, including melatonin suppression and photoperiodic response (Webb et al., 1985; Foster et al., 1991; Freedman et al., 1999; Lucas et al., 1999). Similarly, studies in humans indicated that partial or complete loss of the visual system still allows for normal melatonin suppression and circadian phase-shifting (Czeisler et al., 1995; Ruberg et al., 1996; Lockley et al., 1997; Klerman et al., 2002). Furthermore, it has more recently been shown in rats and nonhuman primates that intrinsically photosensitive retinal ganglion cells (ipRGCs) innervating the suprachiasmatic nucleus (SCN) in the hypothalamus will depolarize in response to light with a peak spectral sensitivity of 482 to 484 nm, even with the blockage of synaptic input from rods and cones (Berson et al., 2002; Dacey et al., 2005). These ipRGCs have been shown to be in the same subset of retinal cells that express melanopsin in rats (Provencio et al., 1998; Hattar et al., 2002). Thus, both the anatomical distribution and physiologic profile of melanopsin-containing cells indicate its centrality to normal circadian photoentrainment (Rollag et al., 2003). Recent studies have shown that transfecting nonphotosensitive cells with the melanopsin gene confers light sensitivity to these cells, which provides compelling evidence that melanopsin is the functional ipRGC photopigment (Melyan et al., 2005; Panda et al., 2005; Qiu et al., 2005). Studies of melanopsin-knockout mice, however, demonstrate that although melanopsin plays an integral role in circadian phototransduction and may contribute to pupillary light reflex, it is not necessarily essential for these nonvisual ocular-mediated responses (Ruby et al., 2002; Panda et al., 2002; Hattar et al., 2003; Lucas et al., 2003). Other studies with animals and humans indicate that the visual rods and cones provide input to the SCN (Aggelopoulos and Meissl, 2000; Mrosovsky, 2003; Figueiro et al., 2004). Further research is needed to clarify the exact role of melanopsin and classic visual photopigments in circadian phototransduction.

Work done on circadian phase-shifting in humans has highlighted the possibility of condition-dependent physiologic variability in circadian response to light, as the melatonin suppression response to 555-nm monochromatic light attenuated over a 6.5-h exposure period while the 460-nm suppression did not (Lockley et al., 2003). Correspondingly, experiments on transgenic rodless, coneless, melanopsin-knockout mice have demonstrated a complete loss of all accessory visual functions measured, including pupillary reflex, light/dark cycle entrainment, and masking response to light, suggesting these known receptors can account for all nonvisual phototransduction (Hattar et al., 2003). This is consistent with the finding that the non-image-forming light response of pupillary constriction in rodless/coneless mice is preserved with a peak spectral sensitivity of 479 nm (Lucas et al., 2001). Furthermore, others have shown the spectral sensitivity of retinally degenerate mice to be different from that of wild-type mice for circadian phase-shifting, suggesting a possible role for the classic visual photopigments in circadian phototransduction (Yoshimura and Ebihara, 1996).

While the human action spectra for melatonin suppression can be interpreted as a single novel photoreceptor mediating phototransduction along the retinohypothalamic tract, studies with transgenic rodent models suggest rod and cone photoreceptors have the capacity to transduce light for circadian regulation (Panda et al., 2002). Aggelopoulos and Meissl (2000) showed that light acting via rod and cone pathways could have state-dependent, opposing actions on the electrical activity of SCN neurons and thus potentially influence the response of these neurons to light stimuli. Using an endpoint of electrical activity as measured by extracellular electrodes, the investigators found that the sensitivity of SCN neurons to light became proportionally higher, with a lower threshold for excitation, with increased time of dark adaptation to scotopic conditions. They also concluded from the significantly divergent sensitivities of SCN neurons to 505-nm light under dark- and light-adapted preconditions that there are separate cone and rod inputs to the SCN and that most SCN neurons receive both types of input. Their extensive findings suggest a complex, dynamic interrelationship between the traditional visual photoreceptors and SCN cell sensitivity to light stimuli, relative to various adaptive lighting conditions (Aggelopoulos and Meissl, 2000).

These rodent studies raised the possibility that the phototransductive physiology of the retinohypothalamic tract in humans might be modulated by the visual rod and cone photoreceptors. Alternatively, subthreshold activation of the circadian photoreceptive system may have the capacity to alter baseline physiology at its receptors without acutely impacting melatonin output downstream. The aim of the following two-part study was to test the hypothesis that dim light adaptation will dampen the subsequent suppression of melatonin by monochromatic light in healthy human subjects.

MATERIALS AND METHODS

Study Design

A complete within-subjects design was used for each of the two experiments. Four hundred sixty nanometers was selected as an optimized wavelength for monochromatic light suppression of melatonin in the peak range of the human action spectrum (Brainard et al., 2001a; Thapan et al., 2001). Two different intensities of 460-nm monochromatic light were tested in two separate experiments: 3.1 $\mu W/cm^2$ and 7.0 $\mu W/cm^2$. These intensities were predicted to be effective in suppressing melatonin, without saturating the response and masking any differences between the adaptive lighting conditions. The adaptive environments used prior to this suppression test were to reflect 1) negligible visual photoreceptor activity, using blindfolding in ambient darkness; and 2) primary cone photoreceptor activity (photopic vision), using 4 cd/m^2 (about 18 lx) dim white light conditions. For reference, typical office illumination is 200 to 300 lx, which is equivalent to a luminance of 12 to 18 cd/m^2. Photopic vision in humans is defined as the vision that is dominated by cone photoreceptors and occurs in lighting conditions at luminances of approximately 4 cd/m^2 and higher (Sliney and Wolbarsht, 1980; Rea, 2000). Additionally, two control study nights were included for each of the two experimental adaptive conditions, in which subjects were exposed to either 3) blindfolding in darkness or 4) 4 cd/m^2 white light for the entirety of the study night. The 2nd experiment, conducted for the purposes of verifying and replicating the significant findings of the 1st experiment, was carried out with a higher intensity of 460-nm monochromatic light (7.0 $\mu W/cm^2$ vs. 3.1 $\mu W/cm^2$) during the melatonin suppression test. This 2nd replication study was performed on a different cohort of subjects. In both experiments reported here, study nights were separated by at least 6 days to exclude any acute disturbance by the study conditions to the subject's usual circadian melatonin rhythm. Additionally, subjects were asked to refrain from caffeine and naps longer than 30 min in duration after 1800 h on the days of their study nights.

Subjects

Eight subjects were used in each of the two experiments. Subjects excluded from the study were those who were involved in shift work, those who participated or planned to participate in long-distance jet travel just before or during the study period, those who had irregular sleeping schedules, and those who were on any medications known to interfere with central neurotransmitter balance or the neurohormonal physiology surrounding the production of melatonin. Included subjects had a stable daily sleeping pattern and passed a physical examination for general and ocular health. Subjects signed an approved institutional review board consent document prior to acceptance into the study. Each experiment included 5 females and 3 males between the ages of 18 and 30 years (mean ± SEM age, 23.6 ± 1.0 years for experiment 1, 25.0 ± 1.0 years for experiment 2). All subjects completed their assigned experiment. The self-reported mean morning wakeup times for cohorts in both experiments were internally consistent without statistically significant variability across study days. The mean wake times were identical between the 2 cohorts of the 2 experiments: 0718 h ± 18 min. Finally, all subjects passed the Ishihara test for normal color vision.

Light Exposure and Melatonin Sampling Protocol

Each study night began at midnight, when subjects' eyes were dilated with 1 drop of 0.5% cyclopentolate HCl in each eye. This pharmacologic dilation helped standardize the physiologic variation in pupillary constriction and thus photic input to the retina during study light exposures. From midnight to just before 0200 h (± 10 min), subjects were exposed to their adaptation condition, which was either to be blindfolded in darkness (for the dark-adaptation periods and dark-control study nights) or 4 cd/m^2 white light (for the photopic-adaptation and photopic-control study nights). During this 2-h period, subjects remained sitting upright for 120 min and were allowed to listen to music with headphones or engage in quiet conversation.

Photopic adaptation with white light at 4 cd/m^2 was delivered using a large, uniform, and patternless ganzfeld dome apparatus. The dome contains a polychromatic white light stimulus from 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), which was fitted with a fiber-optic light guide (model #SI-40-8, Chiu Technical) and aimed at the top of the dome perimeter (Gaddy et al., 1993). Subjects were seated upright with their chins perpendicularly aligned with the edge of the desk upon which the dome rested and their eyes level with the center of the dome. This condition simulated low-intensity white light dim room conditions. At just before 0200 h, as subjects were continually exposed to their adaptation lighting condition without interruption, a 10-mL blood sample was taken by venipuncture of the antecubital vein. Following this 1st blood draw, subjects who were assigned to their control study nights maintained their initial adaptation condition for the remainder of the study night, until 0330 h ± 10 min (with a 90-min exposure time). Alternately, subjects who were assigned to their suppression test study nights were immediately moved in rolling chairs (remaining seated) to an adjacent monochromatic light exposure unit following the 1st blood draw. This unit consists of a separate electronic, optic, and ganzfeld dome exposure array. During this suppression test portion of the experiment, the subject's head rested in an ophthalmologic head holder facing the ganzfeld apparatus with gaze fixed on the center of the dome that encompassed the entire visual field. This equipment and exposure technique are described in greater detail elsewhere (Brainard et al., 2001a; Brainard et al., 2001b). All subjects, regardless of their study condition assignments, were to sit upright with their feet on the floor and eyes open during this portion of the study night. The 2nd blood draw was conducted at 0330 h ± 10 min while subjects were still exposed to their assigned lighting condition. At the conclusion of the 2nd blood draw, subjects were released from the laboratory. Although frequent blood sampling during and after the light exposure would reveal the time course of melatonin suppression and recovery, these issues were not relevant to testing our hypothesis.

Blood samples were collected in glass vacutainers that contained EDTA. Plasma was separated by refrigerated centrifugation, aliquoted into cryogenic vials, and stored at –20 °C until they were assayed. Melatonin concentrations were assayed using antiserum described by Rollag and Niswender (1976). Radiolabled ligand was prepared by adding 100 µL of a pyridine containing 60 nmol 5-methoxytryptamine to 250 µCi (0.1 nmol) Bolton-Hunter reagent (NEN) in approximately 100 µL benzene. The reaction was allowed to proceed for 20 min before adding 500 µL of water. After waiting 5 min to ensure that the unreacted Bolton-Hunter reagent was destroyed, the radioactivity was diluted to approximately 100 cpm/µL with assay buffer (Rollag and Niswender, 1976). Duplicate aliquots of 200 µL of each unknown and control sample were extracted into 2 mL of chloroform. The chloroform was removed in a SpeedVac centrifuge (Savant Instruments, Waltham, MA) and resuspended in 200 µL of assay buffer (phosphate buffered saline, pH 7.4, containing 0.1% gelatin with 100 mg thimerosal/liter as a preservative). The extracts were washed twice with 3 mL of petroleum ether, then evaporated to dryness in a SpeedVac before being resuspended in 200 µL of deionized water. Approximately 50,000 cpm of radiolabled ligand and a 1:256,000 dilution of antiserum (R1055, 9/16/74) were added to each unknown and a triplicate 2-fold geometric series of standards ranging in concentration from 0.2 to 205 pg per 200 µL assay buffer. The final assay volume of buffer in each tube was 400 µL. At the end of the 48-h incubation period, 3 mL of 95% ethanol (4C) was added to each assay tube and the bound radioactivity precipitated by centrifugation at 2000 × g for 30 min. The supernatant was decanted, and radioactivity in the precipitate was quantified. The quantity of melatonin immunoreactivity in the samples was calculated with the use of a computer program (M.L. Jaffe and Associates, Silver Spring, MD; Davis et al., 1980). All solutions were maintained at 4 °C throughout the radioimmunoassay procedure. Assay results were not corrected for recovery (which was proven to be >95% in independent trials). The minimum detection limit of the assay is 0.5 to 2.0 pg/mL (Brainard et al., 2001a).

Statistics

Identical statistical procedures were employed for each of the studies reported here. A two-way, repeated measures ANOVA was used to assess differences in measured melatonin levels over time and between conditions. Then, 2-tailed, paired *t* tests were used to determine significant changes between the pre-exposure

and post-exposure melatonin values of each study night. Percent melatonin change scores were calculated by subtracting the plasma melatonin value at the end of the study night (0330 h) from the value following the adaptation period (0200 h) and dividing this difference by the post-adaptation, pre-exposure (0200 h) plasma melatonin value:

$$\text{Percent Melatonin Change Score} = 100 \times (0330\text{ h melatonin} - 0200\text{ h melatonin}) / 0200\text{ h melatonin}$$

A two-tailed, paired Student *t* test was used to compare these percent melatonin change scores. Importantly, two data points from each subject's endogenous melatonin rhythm, one at 0200 h and one at 0330 h, are used to establish that the study is being done during the period of high nocturnal levels of the subject's melatonin rhythm. This also ensures that the post-adaptation, pre-exposure melatonin value (against which the subsequent suppression response is being contrasted) is in fact sufficiently high enough to detect a potential light-induced melatonin suppression. Without a 0200-h reference point, the 0330-h, post-suppression melatonin value could just as easily be attributed to a subject's endogenously declining melatonin levels as to any effect of light exposure. It is well established that melatonin rhythm is dynamic across the nighttime, and to examine a single light suppression value out of the context of the individual's melatonin rhythm is not biologically meaningful.

To further compare the two adaptation conditions with each other and any difference in their effect on subsequent melatonin suppression, the percent melatonin change scores were normalized to control-adjusted change scores. This adjustment is necessary to account for the normal individual rise or fall in plasma melatonin levels with respect to light-induced changes (Gaddy et al., 1993; Brainard et al., 1997). This was done by subtracting a subject's percent change score on a control study night (either darkness or photopic adaptation lighting throughout the entire study night) from his or her percent change score on the suppression-test study night correlating to that adaptation condition:

$$\text{Control-Adjusted Percent Change Melatonin} = \text{Percent Change Melatonin}_{460\text{ nm exposure night}} - \text{Percent Change Melatonin}_{\text{adaptation control night}}$$

Finally, a two-tailed, paired Student *t* test was used to compare these control-adjusted percent melatonin change scores.

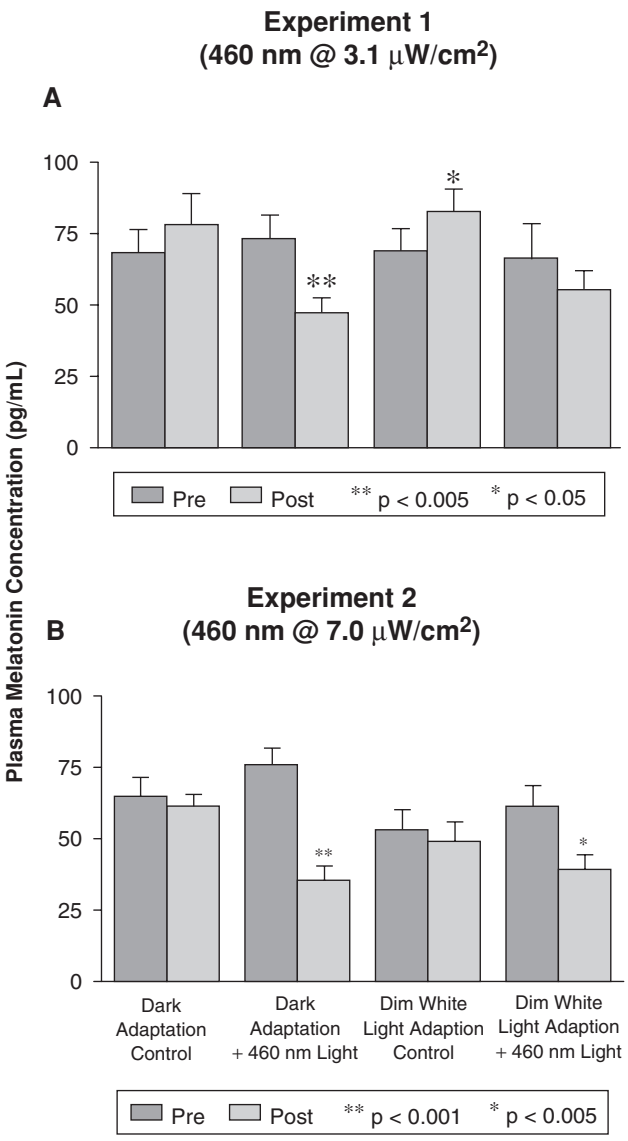

Figure 1. Postadaptation plasma melatonin concentration response to monochromatic light. Plasma melatonin concentration change for 8 healthy human subjects in all 4 experimental conditions at 2 different intensities of 460-nm monochromatic light. As expected, in the dark-adaptation control conditions, melatonin remains high throughout the control study nights but is significantly suppressed by the 460-nm monochromatic light exposure during the exposure nights. On the dim white light (18 lx) control nights, the same high levels of melatonin are seen as in the dark control nights, indicating the inability of white light at this dim level to suppress melatonin. (A) Experiment 1, subjects exposed to 460-nm light at 3.1 μW/cm². (B) Experiment 2, subjects exposed to 460-nm light at 7.0 μW/cm².

RESULTS

In the 1st study, the two-way ANOVAs showed no effect of time of night ($F = 1.78$), no effect of adaptation condition ($F = 0.58$), and a nonsignificant trend

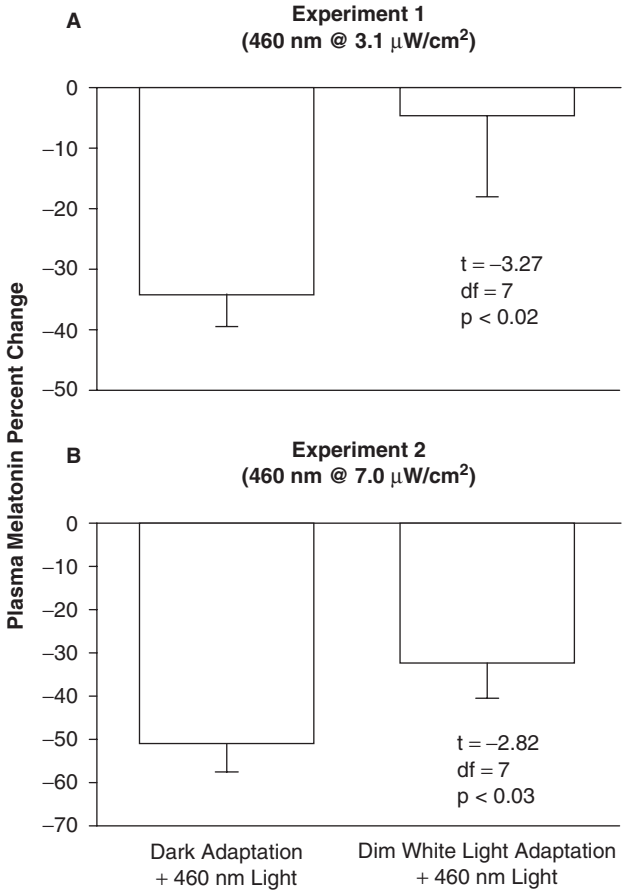

Figure 2. Comparison of percent suppression of melatonin by monochromatic light across adaptation conditions. Percent change in melatonin concentration with 460-nm monochromatic light exposure after dark adaptation and dim white light adaptation. The negative axis is used to portray the downward movement in melatonin concentration that is observed with suppression, portrayed here as a percent of the original, premonochromatic exposure melatonin concentration. (A) Melatonin percent change with 460-nm monochromatic light suppression carried out at an intensity of 3.1 μW/cm². (B) Melatonin percent change with 460-nm monochromatic light suppression carried out at an intensity of 7.0 μW/cm².

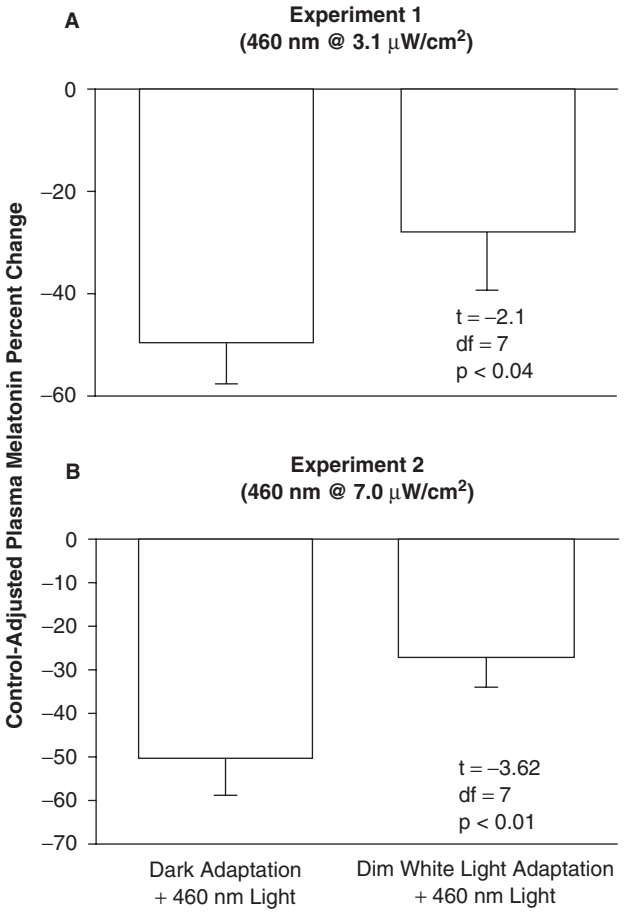

Figure 3. Postadaptation control-adjusted melatonin response to monochromatic light. Control-adjusted plasma melatonin concentration percent change after 460-nm monochromatic light, following 2 h of dark adaptation and dim white light (18 lx) adaptation. (A) Control-adjusted melatonin response to 460-nm monochromatic light suppression carried out at an intensity of 3.1 μW/cm². (B) Control-adjusted melatonin response to 460-nm monochromatic light suppression carried out at an intensity of 7.0 μW/cm².

for an interaction effect ($F = 2.38$, $p = 0.079$). In the follow-up study with a higher 460-nm test stimulus, two-way ANOVA demonstrated no significant effect of time of night ($F = 1.97$), a significant effect of adaptation condition ($F = 17.9$, $p < 0.0001$), and a significant interaction effect ($F = 4.39$, $p < 0.010$). Figure 1 illustrates the mean ± SEM measured melatonin values with results of the paired Student *t* tests.

Mean ± SEM percent change melatonin scores for the two experiments prior to control adjusting are shown in Figures 2A and 2B along with the results of the paired Student *t* tests. As shown in Figure 2, 2-h adaptation to 18-lx white light from midnight to 0200 h resulted in a significant decrease in the magnitude of subsequent melatonin suppression by 460-nm light at both intensities tested (3.1 μW/cm² and 7 μW/cm²), when compared with 2 h of pre-suppression dark adaptation. This result is reflected in Figures 1A and 1B by the blunted melatonin reductions in response to 460-nm exposure after adaptation with dim white light versus dark adaptation.

Furthermore, this difference in statistical significance shown in Figure 2 is maintained when analyzing the average percent change melatonin concentration for each cohort before and after suppression following the two adaptation conditions (Figs. 3A and 3B). For the

460-nm monochromatic light test at 3.1 μW/cm^2, the control-adjusted melatonin percent change was 49.5% ± 8.0% after dark adaptation versus 27.7% ± 8.2% following 18-lx white light adaptation over the same time period (Fig. 2A). Likewise, for the 460-nm monochromatic light test at 7.0 μW/cm^2, the control-adjusted melatonin percent change was 50.5% ± 8.2% after dark adaptation versus 27.3% ± 6.9% following 18-lx white light adaptation (Fig. 2B). Thus, in the 2 different subject cohorts studied, with dim 18-lx adaptation, there were 44% and 46% *decreases* in control-adjusted melatonin suppression at 3.1 μW/cm^2 and 7.0 μW/cm^2 of 460-nm light, respectively, when compared with adaptation to 2 h of darkness prior to suppression.

DISCUSSION

Our results demonstrate that exposure to white light at levels as low as 18 lx for 2 h at night can significantly decrease the magnitude of subsequent melatonin suppression by 460-nm monochromatic light up to 46% in healthy male and female volunteers. Thus, photopic light levels appear to diminish phototransduction as measured by plasma melatonin suppression. This finding supports our original hypothesis based on the observations by Aggelopoulos and Meissl (2000) in rats.

The attenuation of melatonin suppression by preceding adaptation to 18-lx white light occurred with 460-nm monochromatic light at both 3.1 μW/cm^2 and 7.0 μW/cm^2. Interestingly, more than doubling the intensity of monochromatic light used in the melatonin suppression test did not evoke a significantly larger control-adjusted mean suppression for either experiment. In fact, the resultant control-adjusted percent melatonin suppression values for the two parts of the study were almost identical. One explanation for this can be found by examining the 460-nm dose-response curve for melatonin suppression in humans (Brainard et al., 2001a). From this curve, it is evident that both of these intensities of 460-nm light fall within the steep-sloping, pseudo-linear portion of the curve, where there is an exponential rate of change in percent melatonin suppression with increasing photon density. The test intensities on that portion of the curve (2.3, 3.1, 6.9, and 12 μW/cm^2) have a large standard error of the mean (Brainard et al., 2001a). This high variability, combined with normal biological variability in photosensitivity and in the magnitude of circadian melatonin rhythms, could magnify the expected response variation within any cohort to the point of significant overlap in melatonin suppression capacity between photon densities. Thus, selecting an intensity for melatonin suppression that is high enough to allow for measurable contrast, yet not so high as to saturate the novel photoreceptive system to the point of masking any adaptation effects, is a challenge. An additional potentially confounding factor in predicting the correlation between photon density and the level of melatonin suppression is the very process that our study aims to begin elucidating, namely, the effects of adaptation in circadian photoreception. Variability across different groups of test subjects may further multiply the expected overall variability in melatonin suppression response to monochromatic light.

This difficulty in predicting the behavior of a melatonin suppression response in the steep-sloping portion of the dose-response curve for 460-nm monochromatic light melatonin suppression is also likely responsible for the initial finding of no significance relative to adaptation condition in the 2-way, repeated measures ANOVA for experiment 1. Because all other statistical probes used on the data in experiment 1 indicated a significant effect of adaptation, our laboratory felt compelled to repeat this experiment (i.e., experiment 2), using a slightly higher intensity of monochromatic light to gain a greater contrast of suppression against background melatonin levels and overcome the significant variability within the exponential portion of the dose-response curve. This adjustment did in fact produce the statistically significant differences in two-way repeated-measures ANOVA observed in experiment 2, while all other statistical analyses remained significant.

As with any study employing observation of physiologic phenomena in human subjects, it is possible that anomalies and normal biologic variability could lead to false conclusions. For example, if a whole cohort of volunteers had an unusually high melatonin level at 0330 h on their adaptation-control nights, this could lead to the false appearance of a greater suppression response on the 460-nm exposure nights after control adjusting. However, given that our findings are consistent across two studies, the combined likelihood of the observed effect being a result of such randomness is greatly reduced, if not negligible. Furthermore, the 0330-h control melatonin values in the two studies reported here are comparable to numerous other studies published by our laboratory (Brainard et al., 1997; Brainard et al., 2001a).

Overall, from these results, we can conclude that the light stimulus that is capable of dampening the

magnitude of the circadian system's subsequent melatonin suppression response is, alone, not intense enough to stimulate a measurable melatonin response at 0200 h. Thus, with varying histories of light exposure, some silent alterations in baseline physiology are occurring. With these findings, it is possible that a white light stimulus that is subthreshold for melatonin suppression is still strong enough to directly modulate photoisomerase activity within the melanopsin-containing ipRGCs. A recent electrophysiological study showed that background light causes ipRGCs to desensitize (Wong et al., 2005). It is also possible, however, that the visual photoreceptors are involved in modifying circadian phototransduction, leading to the observed reduction in melatonin suppression response. Multiple lines of evidence indicate that rods and cones may be involved in circadian phototransduction (Aggelopoulos and Meissl, 2000; Ruby et al., 2002; Panda et al., 2002; Hattar et al., 2003; Mrosovsky, 2003; Figueiro et al., 2004; Dacey et al., 2005). Similarly, it appears that the relationship may be reciprocal, where in humans it has been shown that a photopigment with a unique, opsin-type action spectrum and a peak sensitivity of 483 nm can drive the adaptation of cone-mediated visual pathway depending on the time of day (Hankins and Lucas, 2002).

There is a well-known dose-response relationship between the intensity of light and the resultant magnitude of melatonin suppression (Lynch et al., 1981; Brainard et al., 1983; Brainard et al., 1988; McIntyre et al., 1989; Aoki et al., 1998; Zeitzer et al., 2000). Much less well-characterized, however, is the effect of light adaptation on circadian regulation. A very early study by Lynch and colleagues (1981) showed that with adaptation, rats could respond to a dim stimulus as either daytime or nighttime in terms of circadian regulation. Additionally, studies in animal models show superlative sensitivity to phase-shifting by light after exposure to extended periods of darkness (Shimomura and Menaker, 1994; Refinetti, 2003). Two human studies show that a higher intensity light history over a given period of time dampens the magnitude of subsequent melatonin suppression by acute light exposure. In one study, one week of controlled dim light (less than 200 lx) resulted in 53% subsequent suppression of melatonin by 500-lx white light compared with only 41% after a week of bright light (5000 to 7000 lx) exposure (Hebert et al., 2002). In another study, a light history of 0.5 lx for more than 63 h prior to a 6.5-h, 200-lx white light exposure resulted in a mean melatonin suppression of 85.7%, compared with 71.2% after a constant light history of 200 lx over an equal period (Smith et al., 2004). These two human studies investigated the relationship between pre-exposure conditions and subsequent melatonin suppression using white light as the probe for the suppression test. In contrast, our study employs the peak spectral sensitivity for human melatonin suppression. Current evidence indicates that monochromatic light in the range of 446 to 477 nm is optimal for targeting the circadian photoreceptor system for suppressing melatonin (Brainard et al., 2001a; Thapan et al., 2001).

Thus, it is possible that circadian phototransduction is more physiologically involved than a single novel circadian photopigment can explain. Because of this unfolding complexity, it is becoming increasingly important to define which conditions comprise the background upon which melatonin suppression tests are being conducted. Only in this way can we distinguish which findings are those of idealized, baseline physiology, and which are compensatory or adaptive to changed environmental settings.

There are two primary neural projections to the SCN from the retina in rats, namely, one involving the intergeniculate leaflet (IGL)—a primary transmission center for neurons carrying information from the visual photoreceptors in the retina to the visual cortex—and one monosynaptic projection through the optic chiasm to the SCN (Groos and Meijer, 1985). In this dual projection system, those connections passing through the IGL are not necessary for entrainment of circadian rhythms but certainly have the potential to control the phase of the circadian oscillator in the SCN (Groos and Meijer, 1985). Accordingly, the rod and cone projections would not be the sole informants to the SCN of the lighting environment at any point in time but would have a means for modulating the background neurochemical synaptic milieu of the SCN upon which the other, more direct retinohypothalamic projections are superimposed. It has been shown that the novel circadian photoreception system has the capacity to drive adaptation of the primary visual cone system according to the time of day (Hankins and Lucas, 2002) and that the melanopsin-expressing ipRGCs in primates project visual-type stimuli to the IGL (Dacey et al., 2005). Thus, regardless of its role in explaining findings presented here, communication between the circadian and visual photoreceptive systems is occurring. It is not clear at this point whether this relationship is mutually reciprocal.

On a more microscopic level, it has been shown that certain mammalian SCN cells have a variable

electrical threshold for response in a fixed direction (excitatory or inhibitory) to a white light stimulus, varying only in the magnitude of their response with little resultant effect upon the overall SCN activity (Groos and Meijer, 1985). This is not absolutely consistent with recent findings in circadian phase shifting in humans (Lockley et al., 2003) or with the findings of our present study. Thus, the spectral quality of the light used in melatonin suppression likely changes the observed, integrated SCN response. How such integration might occur is unknown. Alternatively, in their work with SCN neuron electrical response to photopic and scotopic inputs from classic photoreceptors, Aggelopoulos and Meissl (2000) found that inhibitory or excitatory responses in SCN cells varied based on levels of prolonged prior adaptation before light/dark pulses. There is also a possibility of spectral opponency occurring between two distinct photoreceptive systems. This possibility cannot be ruled in or out by our present findings. According to this hypothesis, two wavelengths present in the white light source, in this case, blue and yellow-orange range wavelengths, would have opposite and canceling physiologic effects, either at the photoreceptor level or further downstream, resulting in negligible change in melatonin output (Figueiro et al., 2004). To date, evidence does not yet favor any one of these possible explanations over another for the observed findings in circadian phototransduction.

Practically speaking, the use of focused light exposure in humans for clinical therapy (e.g., seasonal affective disorder) or for realigning the circadian system in shift work, jet travel, or space exploration is likely to occur as a superimposition upon a background level of environmental lighting exposure. Thus, the significant modulation of melatonin suppression by a history of dim white light in our findings has important implications to any scientific effort toward optimizing lighting for therapeutic and/or productivity purposes. It is highly unlikely that any individual will present himself or herself to a focused lighting countermeasure without a recent history of varied polychromatic light at levels well above 18 lx.

CONCLUSION

Our results indicate that the activity of the novel circadian photoreceptive system is subject to subthreshold modulation of its sensitivity to subsequent monochromatic light exposure, varying with the conditions of light adaptation prior to exposure. The physiology for this modulation and the identity of its source are yet to be fully characterized, but these results highlight the importance of accounting for light exposure history when attempting to predict the behavior of circadian phototransduction. Characterizing the phototransductive effects of everyday background lighting, and thus accounting for its impact upon light transmission targeting circadian processes, is elemental to the goal of harnessing light to its fullest capacity for positive influence upon human health and performance.

ACKNOWLEDGMENTS

Preliminary reports of these data were given at the American Society for Photobiology (2002) and European Pineal and Biological Rhythms Society (2002) in abstract and oral presentation formats. The authors thank Nick Leone, Adam Perry, Gena Glickman, Jeff Greeson, Jim Gardner, Brenda Byrne, Marielle Stone, Benjamin Warfield, and Robert Fucci for their faithful and conscientious technical assistance in carrying out the involved protocol of this study. The authors also thank their skilled phlebotomists Christine Glasgow, Robert Glasgow, Patricia Becerra, Marianne Hamel, Kareema Brown, and Demeka Bethune as well as nurses from Thomas Jefferson University Hospital Emergency Department. Additional thanks to Benjamin Warfield for his assistance in preparing the final graphs and article. This work was supported in part by grants from the National Space Biomedical Research Institute under NASA Cooperative Agreement HPF.002.08, the National Institute of Neurological Disorders and Stroke NIH RO1NS36590, and the Philadelphia Section of the Illuminating Engineering Society.

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