# Short-Wavelength Sensitivity for the Direct Effects of Light on Alertness, Vigilance, and the Waking Electroencephalogram in Humans

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**Study Objectives:** To assess the wavelength-dependent sensitivity of the acute effects of ocular light exposure on alertness, performance, waking electroencephalogram (EEG), and cortisol.

**Design:** A between-subjects design was employed to compare the effects of exposure to 460-nm or 555-nm light for 6.5 hours during the biological night.

**Setting:** Intensive Physiological Monitoring Unit, Brigham and Women's Hospital, Boston, MA.

**Patients and Participants:** Sixteen healthy adults (8 women; mean age ± SD = 23.3 ± 2.4 years).

**Interventions:** Subjects were exposed to equal photon densities (2.8 x $10^{13}$ photons• $cm^{-2}$• $s^{-1}$) of either 460-nm (n = 8) or 555-nm (n = 8) monochromatic light for 6.5 hours, 15 minutes after mydriasis.

**Measurements and Results:** Subjects underwent continuous EEG/electrooculogram recordings and completed a performance battery every 30 to 60 minutes. As compared with those exposed to 555-nm light, subjects exposed to 460-nm light had significantly lower subjective sleepiness ratings, decreased auditory reaction time, fewer attentional failures, decreased EEG power density in the delta-theta range (0.5-5.5 Hz), and increased EEG power density in the high-alpha range (9.5-10.5 Hz). Light had no direct effect on cortisol.

**Conclusions:** Short-wavelength sensitivity to the acute alerting effects of light indicates that the visual photopic system is not the primary photoreceptor system mediating these responses to light. The frequency-specific changes in the waking EEG indicate that short-wavelength light is a powerful agent that immediately attenuates the negative effects of both homeostatic sleep pressure and the circadian drive for sleep on alertness, performance, and the ability to sustain attention.

**Keywords:** Alertness, alpha waves, auditory performance, circadian photoreception, cortisol, fatigue, light, vigilance, waking EEG, light wavelength

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## INTRODUCTION

OCULAR EXPOSURE TO VISIBLE LIGHT HAS A RANGE OF NEUROBIOLOGICAL EFFECTS IN HUMANS, INCLUDING RESETTING THE ENDOGENOUS CIRCADIAN pacemaker,[1] acute suppression of pineal melatonin production,[2] elevation of core body temperature[3] and heart rate,[4,5] and stimulation of cortisol production in the early morning.[6] Exposure to broad-spectrum white light at night has also been shown to have acute, dose-dependent alerting effects, as measured by subjective sleepiness ratings, improved psychomotor vigilance reaction times and reduced lapses, reduction of attentional failures as indicated by electrooculogram-derived slow rolling eye movements, and suppression of theta-alpha (5-9 Hz) activity in the waking electroencephalogram (EEG).[5,7-14] More recently, 5 hours of white-light exposure during the day (noon-5:00 PM) has also been shown to enhance alertness and performance and reduce the incidence of slow eye movements,[15] in contrast to previous reports.[8,16]

The photoreceptor system or systems and neuroanatomic pathways mediating these responses are yet to be fully elucidated, although recent advances indicate that a novel nonrod, noncone photoreceptor system contributes significantly to these effects in nonhuman mammals.[17,18] Complementary studies in humans have suggested that neither functional rods nor functional cones are required for positive melatonin-suppression responses in color-blind subjects[19] or melatonin suppression and circadian phase shifting in some totally blind individuals.[20,21] Action spectra for melatonin suppression following short-duration (30- to 90-minute) monochromatic light exposure[22,23] or the latency of the cone-driven electroretinogram b-wave response following light adaptation[24] reveal a short-wavelength peak in wavelength-dependent sensitivity ($\lambda_{max}$ 446-483 nm) that does not match those of the 3-cone photopic ($\lambda_{max}$ 555 nm) or rod scotopic ($\lambda_{max}$ 510 nm) visual systems, consistent with the hypothesis that a novel nonrod, noncone photoreceptor system mediates, at least in part, these responses, as in other mammals[17,25] ($\lambda_{max}$ 472-482 nm). Similarly, wavelength sensitivity for photic resetting of the human circadian pacemaker is also blue shifted relative to the visual 3-cone photopic system for both phase delay[26] and phase advance[27,28] shifts, and, recently, it has been shown that acute short-duration (2 hour) elevation of alertness, core body temperature, and heart rate are also short-wavelength sensitive.[29] A novel opsin, melanopsin, is

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present in the mammalian eye,[30] including the human eye,[31] and is present in intrinsically photosensitive retinal ganglion cells (ipRGC) that respond directly to light with a peak spectral sensitivity in the short-wavelength range (~480 nm).[32-34] Rods, cones, or melanopsin are not required to mediate these photic responses, and there are differences in the relative sensitivity to nonvisual effects of light in rodless/coneless animals versus melanopsin-knockout strains, which suggest specific, nonredundant roles for the visual and nonvisual photoreceptor systems.[17,18,35,36] Simultaneous removal of rods, cones, and melanopsin, however, abolishes all visual and nonvisual photic responses.[17,18]

The aim of the current study was to test the wavelength-dependent sensitivity of long-duration light exposure on subjective and objective correlates of arousal. Specifically, we aimed to test the hypotheses that nighttime exposure to 460-nm monochromatic light would preferentially reduce subjective sleepiness, decrease auditory reaction time, decrease auditory lapses, enhance EEG-correlates of alertness, and increase plasma cortisol compared with an identical exposure to an equal photon density of 555-nm monochromatic light.

## METHODS

### Subjects and Prestudy Conditions

We studied 16 healthy subjects (8 women; mean age ± SD = 23.3 ± 2.4 years; range 19-27 years) in the Intensive Physiology Monitoring Unit of the Brigham and Women's Hospital between October 2001 and June 2002. The study was approved by the Human Research Committees at Brigham and Women's Hospital and Thomas Jefferson University, and subjects gave written informed consent prior to study. All had comprehensive physical, psychological and ophthalmologic exams, including an Ishihara color blindness test. For at least 3 weeks prior to entering the Intensive Physiology Monitoring Unit, subjects maintained a self-selected, constant 8-hour sleep/rest/dark schedule confirmed with calls to a time- and date-stamped voicemail at bedtime and wake time for 3 weeks and with actigraphy (Actiwatch-L, Minimitter, Inc., Bend, OR) for at least 7 days prior to entering the unit. Subjects were asked to refrain from use of any prescription or nonprescription medications, supplements, recreational drugs, caffeine, alcohol, or nicotine. Compliance with these instructions was verified by urine and blood toxicology during screening and urine toxicology upon entry to the unit.

### Study Protocol

The study protocol and some study conditions have been described elsewhere.[26,37] Subjects were studied for 9 days in an environment free of time cues (no access to windows, clocks, watches, live TV, radio, internet, telephones, and newspapers and continually supervised by staff trained not to reveal information about the time of day). The schedule consisted of a 3-day baseline (8-hour:16-hour sleep-wake cycle based on average sleep times in the 7 days prior to study entry), an initial 50-hour 10-minute constant routine, a 16-hour light-exposure day, and a second 29-hour 50-minute constant routine, each preceded and followed by an 8-hour sleep episode (Figure 1). During the constant-routine episodes, subjects were asked to remain awake while supervised in constant dim light in a semirecumbent posture, with daily nutritional intake divided into hourly portions (150 mEq Na+/100 mEq K+ (± 20%) controlled nutrient, isocaloric [basal energy expenditure x 1.3] diet, 2000 mL fluids/24 hours).

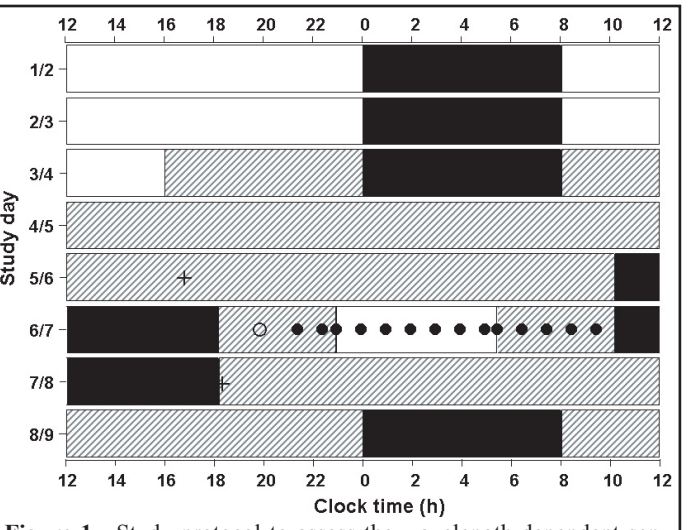


**Figure 1—**Study protocol to assess the wavelength-dependent sensitivity of the acute alerting effects of ocular light exposure. Study days are plotted on the ordinate axis and 24 hours of clock time on the abscissa (12:00 - 12:00 h). Following a 3-day baseline with scheduled sleep (black bars) timed at each subjects' previous 7-day average (average bedtime ± SD = 0:07 ± 1:07 hours), subjects underwent a ~50-hour constant routine (Days 5-6) and 8-hour recovery sleep before the light-exposure 'day' (Days 6-7). Subjects were exposed to monochromatic light for 6.5 hours, centered in the 16-hour waking episode between the constant routines (white bar on Days 6-7). Following the light-exposure 'day,' subjects slept for 8 hours prior to beginning a second constant routine (~30 h) (Days 7-8), followed by a further 8-hour sleep opportunity before discharge. Ambient room lighting during scheduled wake episodes on baseline days 1-3 was ~190 lux maximum (white bars) until midway through the third baseline day, when ambient lighting was maintained at < 2 lux until the end of the study (stippled bars). A performance battery including the Karolinska Sleepiness Scale (KSS), 10-minute auditory psychomotor vigilance test (PVT-10A), and 3-minute (eyes open) Karolinksa Drowsiness Test (KDT) was completed every 60 minutes during the constant routines (not shown) and every 30 to 60 minutes during the light exposure 'day' (● KSS/PVT-10A/KDT; ○ PVT-10A only). Cortisol was assayed from plasma samples taken every 20 to 30 minutes from 30 hours before until 12 hours after the monochromatic-light exposure (+).

During the first 2.5 baseline days, maximum ambient light during scheduled wake was 48 μW/cm$^2$ or ~190 lux when measured vertically at a height of 187 cm and ~88 lux when measured horizontally (137 cm). Midway through day 3, maximum ambient light was decreased to < 2 lux (0.4 μW/cm$^2$ or ~1.5 lux) when measured vertically and ~0.6 lux when measured horizontally and maintained at that level for the remainder of the study. Ambient light was switched off during monochromatic light exposure and scheduled bedrest episodes. Room lighting was generated using ceiling-mounted 4100K fluorescent lamps (F96T12/41U/HO/EW, 95W; F32T8/ADV841/A, 32W; F25T8/TL841, 25W; Philips Lighting, The Netherlands) with digital ballasts (Hi-Lume 1% and Eco-10 ballasts, Lutron Electronics Co., Inc., Coopersburg, PA) transmitted through a UV-stable filter (Lextran 9030 with prismatic lens, GE Plastics, Pittsfield, MA). Routine illuminance and irradiance measures were conducted using an IL1400 radiometer/powermeter with an SEL-033/Y/W or SEL-033/F/W detector, respectively (International Light, Inc., Newburyport, MA).

### Monochromatic Light Exposure

Monochromatic light exposure occurred on Day 6 (Figure 1), and the 6.5-hour exposure was timed to start 9.25 hours before respective wake time during each subjects' baseline days, corresponding on average to approximately 6.75 hours before core body temperature minimum, a phase at which white-light exposure induces robust melatonin suppression, phase-delay shifts, and acute alerting effects.[13,37] Monochromatic light was generated using a 1300-W xenon arc lamp and grating monochromator and administered via a modified Ganzfeld source coated with 96% to 99% reflective paint [see reference 22 for further details]. The monochromatic-light wavelength was confirmed using a PR-650 SpectraScan Colorimeter with a CR-650 cosine receptor (Photo Research Inc., Chatsworth, CA). Routine power measures were conducted using an IL1400 radiometer/powermeter with an SEL-033/F/W detector (International Light, Inc.) and fixed at the front of the dome at approximate eye level using a clear plastic holder.

Subjects were randomly assigned to exposure to either 460-nm (n=8) or 555-nm (n=8) monochromatic light (±10 nm half-peak bandwidth). The target irradiance at the level of the eye was 10.0 $\mu W/cm^2$ and 12.1 $\mu W/cm^2$ for 555 nm and 460 nm, respectively, generating an equal photon density of 2.8 x $10^{13}$ photons· $cm^{-2}$· $s^{-1}$ for both exposures. The measured values, averaged between the start and end of each 90-minute fixed-gaze episode, were 9.9 $\mu W/cm^2$ (555 nm) and 11.8 $\mu W/cm^2$ (460 nm). Ninety minutes prior to and throughout the light exposure, subjects were seated, and, 15 minutes prior to exposure, a pupil dilator was administered to each eye (ophthalmologic preparation of 0.5% cyclopentolate hydrochloride, 1 drop per eye; Cyclogel, Alcon, TX), after which subjects wore black-out goggles until the start of the light exposure. During monochromatic-light exposure, subjects were supervised continually and asked to maintain a fixed gaze for 90 minutes in the Ganzfeld dome before a free gaze for 10 minutes while remaining seated. This sequence was repeated throughout the exposure. During free gazes, eye level irradiance was approximately 1 $\mu W/cm^2$. One individual had an extended free gaze lasting 40 minutes, starting 3 hours and 20 minutes into the 555-nm monochromatic light exposure.

### Sleepiness and Performance Assessments

Subjective sleepiness was rated using the Karolinksa Sleepiness Scales (KSS),[38] a 9-point scale from 1—"very alert" to 9—"very sleepy, fighting sleep." Subjects completed the KSS by pressing the appropriate number on a computer keyboard when prompted, except during monochromatic-light exposure when they responded verbally after having been read the identical instructions and options presented during the visual responses. During the light-exposure day, the KSS was presented every 10 to 20 minutes for the first 90 minutes awake during a sleep-inertia test battery (data not included) and every 30 to 60 minutes through the 16-hour wake episode, including the start of the monochromatic-light exposure, every subsequent hour, and immediately upon lights off. Performance was assessed every 30 to 90 minutes (Figure 1) using an auditory 10-minute psychomotor vigilance task (PVT-10A), in which an auditory signal was presented at random intervals (1-9 seconds) and the subject was asked to press a button as soon as possible after hearing the sound. No simultaneous visual stimulus was presented.

### Waking EEG Recordings

Polysomnographic recordings were made continuously throughout the constant routine and light-exposure episodes using a portable, modular, battery-operated, ambulatory, digital polysomnographic recorder (Vitaport-3 digital recorder, TEMEC Instruments B.V., Kerkrade, The Netherlands). Recordings consisted of EEG, electrooculogram, and a 2-lead electrocardiogram. Electrodes were positioned according to the International 10-20 System, with linked mastoid references (Ax) used for wake recordings from the z-line, Fz-Ax, Cz-Ax, Pz-Ax, and Oz-Ax. Only data from the Cz-Ax derivation (central position on the nasion-inion midline) are presented in this report. All EEG signals were high-pass filtered (time constant: 0.33 seconds), low-pass filtered (-6 dB at 70 Hz, 24 dB/octave), and digitized (resolution: 12-bit, sampling rate: 256 Hz, storage rate: 128 Hz). The raw signals were stored on a Flash RAM Card (SanDisk, Sunnyvale, CA) and downloaded off-line. Electrode impedances were checked using a GRASS F-EZM4 impedance meter (Grass-Telefactor, Astro-Med, Inc., West Warwick, RI) at the beginning and end of the light exposure and every 8 hours throughout the constant routine. Electrode impedances were documented, and electrode applications were repeated until the impedances were all < 10 kΩ. Subjects were also asked to complete the Karolinska Drowsiness Test (KDT) hourly throughout the constant-routine and light-exposure episode, after completing the alertness and performance battery. During the KDT, subjects were instructed to relax and fixate on a 5-cm black dot 1-m away attached to a computer screen for 3 minutes with their eyes open. During the monochromatic-light exposure, subjects were asked to focus on a 30-mm spot at the back of the modified Ganzfeld source approximately 20 cm from eye level.

### Hormone Measurement

Plasma was drawn through an indwelling cannula in a forearm vein and kept patent via a heparinized saline infusion (5 IU heparin/mL 0.45% NaCl, infused at 42 mL/h). Blood samples were transferred to ethylenediaminetetraacetic acid tubes and kept in ice before centrifugation (2200-2800 rpm, 2°C), pipetted into plastic tubes, and stored at -20°C. Plasma melatonin and cortisol was sampled every 30 minutes from Day 3 and every 20 minutes during monochromatic-light exposure. In the current analysis, only cortisol samples from a 48-hour episode were assayed beginning 30 hours prior to onset of monochromatic light exposure, during light exposure, and for 12 hours after light offset (Figure 1). Plasma was not drawn in 2 subjects during light exposure (460 and 555 nm, respectively), and the data were therefore excluded from the cortisol analysis. Plasma cortisol was measured by direct radioimmunoassay using a technique adapted from Riad-Fahmy and colleagues [39] (Stockgrand, Ltd., University of Surrey, UK). The interassay coefficients of variation were 12.8%, 12.2%, and 15.7% for 120.6, 622.0, and 981.8 nmol/L, respectively. Plasma or salivary melatonin was assayed using radioimmunoassay (ALPCO Diagnostics, Salem, NH). Plasma intraassay and interassay coefficients of variation were < 9% and < 11%, respectively, at 1.94 and 16.59 pg/mL. Saliva intraassay and interassay coefficients of variation were < 15% and < 16%, respectively, at 1.65 and 16.57 pg/mL.[26]

### Data Analysis

The waking EEG signals derived from Cz/Ax during the KDT were visually inspected, and 2-second epochs containing muscle artifact, eye blinks, and eye movements were discarded from further analysis. Artifact-free 2-second epochs were subjected to off-line spectral analysis using a fast-Fourier transformation and a 10% cosine window. Data were reduced by discarding spectra above 20 Hz. Since absolute power density varies greatly among individuals, values were expressed for each subject and light condition as a percentage of power density during dim light (< 2 lux) during an interval of equal clock time in the constant routine on the day prior to the light exposure. Log-transformed power densities were compared between the 460-nm and 555-nm monochromatic-light exposures with unpaired t-tests.

Raw KSS ratings and performance parameters (mean and median reaction time, lapses [responses > 500-ms]) measured during monochromatic-light exposure (0-6.5 hours inclusive, Figure 1) were subjected to 2-way analysis of variance with time and wavelength as factors (SAS Institute, Cary, NC).

Cortisol enhancement was calculated from the percentage difference in the area under the curve (AUC), calculated using the trapezoidal method, between the cortisol profiles during the light exposure compared with the corresponding clock times during the previous cortisol cycle on the first constant routine, as described previously for analysis of melatonin suppression.[26] For 1 subject in the 460-nm condition, cortisol AUC was expressed relative to the corresponding clock time 48 hours previously due to missing data. In addition, the 6.5-hour monochromatic-light exposure was divided into 4 quartiles (Q1-4): the first 3 were 100 minutes in duration (90 minutes fixed gaze plus 10 minutes free gaze), and the fourth was 90 minutes long (fixed gaze only). Cortisol AUCs during the quartiles of the light exposure were subjected to 2-way analysis of variance with time and wavelength as factors (SAS Institute). Due to insufficient data, 1 subject was excluded from the analysis of AUC for the 460-nm exposure, and 2 subjects, 1 from each condition, were excluded from the quartile analysis.

## RESULTS

### Subjective Sleepiness

There was no significant difference in KSS ratings between the 2 groups (460 nm, n = 8; 555 nm, n = 7) when assessed at the onset of light exposure (Time 0) (p > .05, unpaired t-test). As shown in Figure 2A, subjective sleepiness ratings remained constantly low throughout the 6.5-hour exposure to 460-nm monochromatic light and were significantly lower than during exposure to an equal photon density of 555-nm monochromatic light (p < .0001). There was no significant effect of time (p = .34) during the exposure, however, or between time and wavelength (p = .83). Sleepiness ratings were maintained at approximately the same level as during the light exposure for up to an 1 hour after the monochromatic exposures ended, but both groups exhibited an increase in sleepiness after that time coincident with the circadian nadir in alertness.

### Auditory Psychomotor Vigilance Test

There was no significant difference in the performance variables measured between the 2 groups (460 nm, n = 8; 555 nm, n = 7) during the first light-exposure test (Time 0) (p > .05, unpaired t-test). As illustrated in Figure 2B, mean auditory reaction times were significantly faster consistently throughout during the 6.5-hour exposure to 460-nm monochromatic light than during exposure to an equal photon density of 555-nm monochromatic light (p < .0001). There was no significant effect of time (p = .98) or between time and wavelength (p = .92). A similar effect was also observed for median auditory reaction time (data not shown) (wavelength, p < .001; time, p = .99; wavelength x time, p = .96). Auditory lapses, depicted in Figure 2C, were also significantly reduced during exposure to 460-nm as compared with 555-nm light (p < .0001) and remained at a low level (< 4 per 10-minute

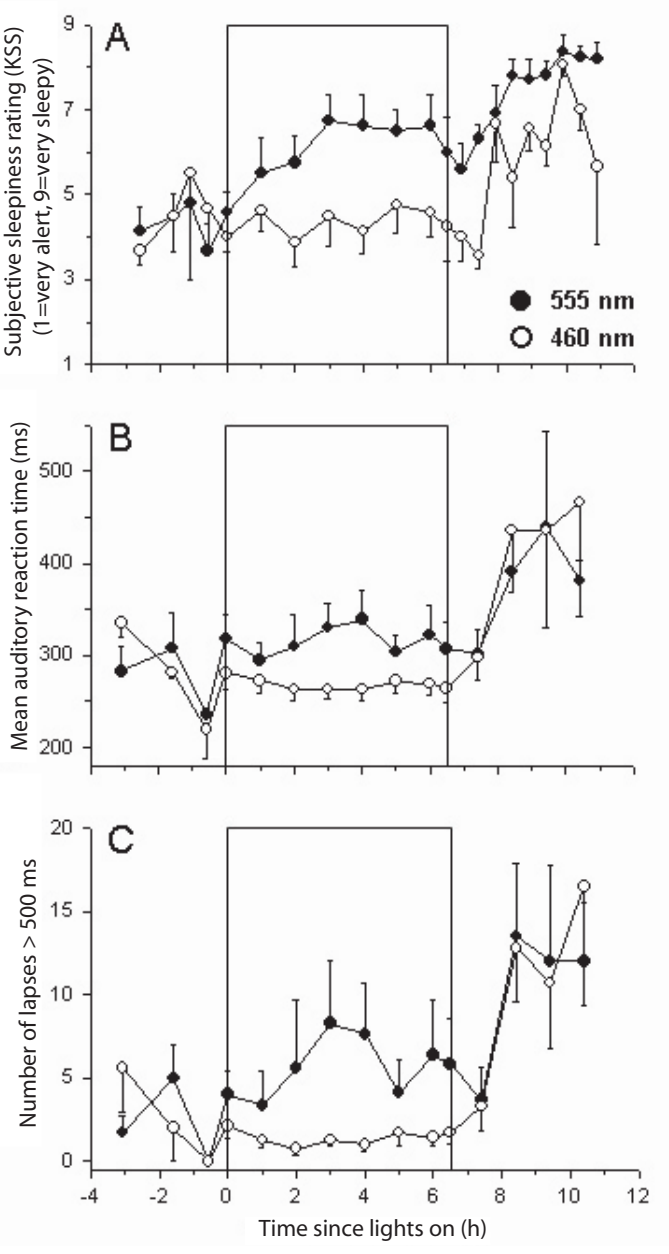


**Figure 2—**Figure 2 shows the mean (± SEM) subjective sleepiness and auditory performance profiles before, during, and after exposure to monochromatic light for 6.5 hours. Exposure to 460-nm monochromatic light (○) significantly improved subjective sleepiness ratings (Figure 2A), mean auditory reaction time (Figure 2B), and auditory lapses of attention (Figure 2C) during the light exposure (0-6.5 h) as compared with subjects exposed to an equal photon density of 555-nm monochromatic light (●) (p < .0001; analysis of variance).

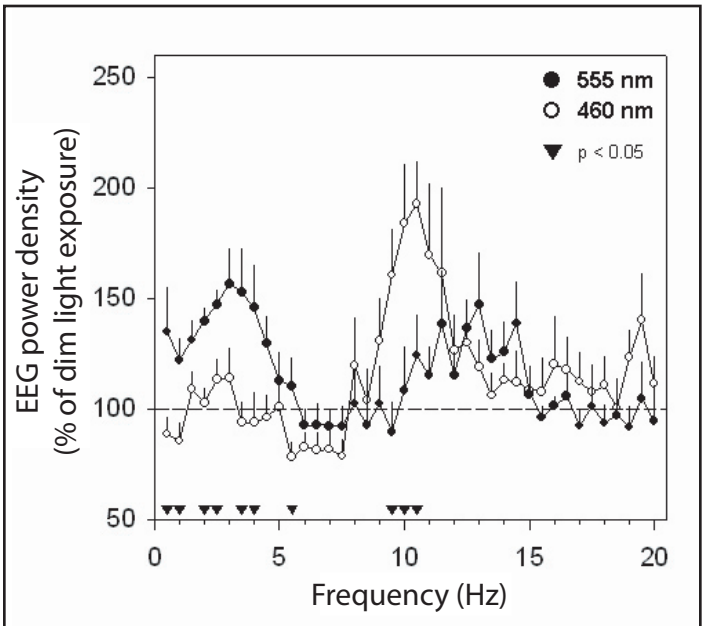


**Figure 3—**Electroencephalogram (EEG) power density mean (+ SEM) during exposure to an equal photon density of either 460-nm (○, n=8) or 555-nm (●, n=8) monochromatic light for 6.5 hours during the biological night. EEG power densities were measured during Karolinksa Drowsiness Test (KDT) episodes, completed every 60 minutes during the monochromatic light exposure. Data are expressed as a percentage of the power density during exposure to continuous dim white light (< 2 lux) for corresponding clock times on the first day of the 50-hour constant routine (i.e., baseline, dotted line). Differences between 460-nm and 555-nm exposures in individual 0.5-Hz frequency bins are indicated by filled triangles ($p < .05$; unpaired t-test). Deviations from baseline ($p < .05$; paired t-tests) were found for the 460-nm condition at 5.5 Hz, 7.5 Hz, 9.5-11 Hz, and 12.5-13 Hz and for the 555-nm condition at 1.5-4.0 Hz, and 12.5-13.0 Hz.

test) without changing through the exposure (time, $p = .95$; wavelength x time, $p = .85$). As with subjective sleepiness ratings, the differences in psychomotor performance parameters persisted for up to an hour before converging and exhibiting a circadian-related deterioration.

### Waking EEG

Exposure to light at 460 nm resulted in frequency-specific changes in the waking EEG, as compared with exposure to 555 nm (Figure 3). Specifically, during exposure to 460 nm, power densities in most bins in the frequency range of 0.5 to 5.5 Hz were reduced, and power densities in the 9.5- to 10.5-Hz range were increased ($p < .05$, unpaired t-tests) (Figure 3).

### Plasma Cortisol

All subjects had an elevated cortisol level in the 90 minutes prior to onset of light exposure compared with the corresponding clock time on the previous day (range 134% to 520%, Figure 4), which was statistically significant for both the 460-nm (mean percentage ± SD = 310 ± 1678%, n = 6, $p < .01$ paired t-test) and 555-nm group (299 ± 95%, n = 7, $p < .01$).

There was no significant difference in cortisol AUC, expressed as percentage of that during the first constant routine, between the two groups (460 nm, n = 6; 555 nm, n = 7) when assessed during the 90 min prior to lights on ($p = .89$, unpaired t-test) (Figure 4). There was a significant effect of time during monochromatic light

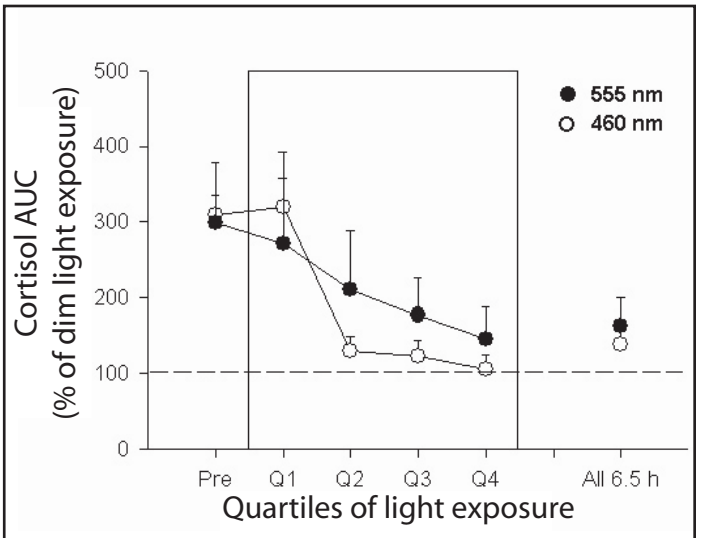


**Figure 4—**The mean (+ SEM) cortisol area under the curve (AUC), before and during exposure to monochromatic light for 6.5 hours, is shown. Data are expressed as a percentage of the AUC during exposure to continuous dim white light (< 2 lux) for corresponding clock times on the previous day (dotted line). There was no difference in cortisol AUC between exposure to 460-nm monochromatic light (○; n=6) and exposure to an equal photon density of 555-nm monochromatic light (●; n=7). Both groups showed a 3-fold increase in cortisol in the 90 minutes prior to experimental light exposure, compared with the corresponding time on the previous day.

exposure ($p = .003$), but no effect of wavelength ($p = .58$) and no interaction between time and wavelength for the cortisol AUC ($p = .50$; 460 nm, n = 6; 555 nm, n = 6) (Figure 4).

### Plasma Melatonin

As we had hypothesized, exposure to 6.5 h of 460-nm monochromatic light caused a significantly greater suppression of melatonin (87.7 ± 11.0%; n=7) compared with 555-nm monochromatic light (39.1 ± 34.1%; n=8) ($p = .002$, one-tailed t-test).[26] Comparison of the respective AUC data showed that, across all subjects with complete data sets for the respective variables, a greater degree of melatonin suppression was positively correlated with lower mean ($r^2 = 0.36$; $p = .02$) and median reaction time ($r^2 = 0.32$; $p = .04$), fewer lapses of attention ($r^2 = 0.49$, $p = .01$)(n = 14), and reduced subjective sleepiness ratings ($r^2 = 0.27$, $p = .06$) (n = 14). Of the EEG frequency ranges that differed between the 460-nm and 555-nm monochromatic exposures (0.5-5.5 Hz and 9.5-10.5 Hz), there was a significant negative correlation between the percentage of melatonin suppression and the percentage increase in delta-theta frequencies (0.5-5.5 Hz) during the light exposure ($r^2 = 0.36$, $p < .05$) but no relationship between melatonin suppression and high-alpha frequency (9.5-10.5 Hz) elevation ($r^2 = 0.07$, $p = .34$) (n = 15). There was also no correlation between the percentage of melatonin suppression and percentage of cortisol enhancement ($r^2 = 0.08$, $p = .34$) (n = 14).

## DISCUSSION

Exposure to 460-nm monochromatic light for 6.5 hours during the biological night significantly decreased subjective sleepiness, improved auditory performance, decreased waking EEG power density in the delta-theta frequency range, and increased power density in the high-frequency alpha range, compared with expo-

sure to an equal photon density of 555-nm monochromatic light. Our findings indicate that the acute alerting effects of ocular light exposure are wavelength dependent and exhibit greater sensitivity to short wavelengths in the visible spectrum. We did not, however, detect a corresponding wavelength-dependent increase in plasma cortisol levels during light exposure under these experimental conditions.

The previous studies that have assessed the acute alerting role of white light have not addressed the photobiological processes mediating this effect: One aim of this study was to establish the wavelength-dependent sensitivity of these photic responses as a first step in that process. Since a greater response was elicited following exposure to an equal number of photons of 460-nm light, as compared with 555-nm light, the current study demonstrates that the photoreceptor or photoreceptors mediating the acute effects of light on subjective and objective correlates of alertness are blue shifted relative to the visual photopic system. Although 460-nm light was more effective at improving alertness and performance, it appears that the long-duration 555-nm light exposure may have had some effect but at a reduced magnitude, as indicated by the rapid deterioration in fatigue an hour after the monochromatic exposures ended (> 6.5 hours; Figure 2). This finding is not consistent with those of Cajochen and colleagues,[29] who were unable to detect a difference in subjective sleepiness between subjects exposed to 550-nm light for 2 hours versus a no-light control. If confirmed, our observation would suggest that the cones may contribute in some part to the acute alerting effects of light, as suggested for circadian-phase resetting and melatonin-suppression responses.[26]

The neurophysiology that mediates the capacity of light to enhance alertness is not fully understood. It is known that intrinsically photosensitive retinal ganglion cells project to a range of targets, including the suprachiasmatic nuclei, subparaventricular zone, and the pretectal area that are implicated in mediating non-image-forming responses, such as phase shifting and pupillary reflex response.[40-42] Furthermore, these cells also project directly to the ventrolateral preoptic area,[42] a hypothalamic nucleus lateral to the optic chiasm and rostral to the suprachiasmatic nuclei that also receives secondary afferents from the suprachiasmatic nuclei, subparaventricular zone, and dorsomedial hypothalamus.[42,43] Ventrolateral preoptic area efferents inhibit the ascending arousal system and may therefore mediate transitions between sleep and wake states.[44] Direct photic input to this nucleus may therefore alter ventrolateral preoptic area activity and waking arousal levels.[42]

An alternate hypothesis suggests that the acute alerting effects of light exposure reduces sleepiness indirectly through acute suppression of melatonin, a hormone closely associated with endogenous sleep propensity and increases in sleepiness if taken exogenously.[45-47] In the current study, increased melatonin suppression was associated with greater arousal, as indicated by reduced sleepiness, improved performance, and a reduction in EEG delta-theta power (0.5-5.5 Hz). The dose-dependent function for melatonin suppression and the acute alerting effects of light are also highly correlated for white-light exposure.[13] This theory, however, cannot explain the recent observation of light-induced performance improvements in the daytime,[15] when circulating levels of melatonin are undetectable.

Previous studies that have measured the acute effects of white-light exposure on EEG power density analysis have shown the major effect to be a suppression of activity in the theta-alpha range (5-9 Hz).[13] EEG power density in the delta and theta frequency ranges is influenced by both circadian and homeostatic processes.[48-51] In the present study, subjects had only one 8-hour sleep opportunity within the 48 hours prior to the experimental light exposure and, thus, were expected to be under higher homeostatic sleep pressure, as compared with the dim-light condition during the corresponding clock time in the first half of the first constant routine that served as the reference and before which subjects had two 8-hour sleep opportunities in the prior 48 hours. The anticipated difference in sleep pressure between these conditions can explain the increase of EEG power density in the delta-theta frequencies relative to baseline observed in the 555-nm condition (Figure 3). Consequently, the suppression of power density in this frequency range by short-wavelength light appears to be mediated, at least in part, by an opposition of sleep homeostatic mechanisms. High-frequency alpha activity (9.25-12.0 Hz), by contrast, does not exhibit a powerful sleep-wake-dependent (i.e., homeostatic) component.[49] Furthermore, given that it has previously been shown to correlate negatively with the circadian changes in subjective sleepiness and circulating plasma melatonin levels,[49] high-frequency alpha activity has been proposed as a specific marker of the endogenous circadian drive for alertness.[49] Our current study showed that short-wavelength light selectively enhanced high-frequency alpha activity, compared with 555-nm light, suggesting that the effects of light might be mediated through inhibition of the circadian drive for sleep during the biological night. Whether this inhibition is achieved through suppression of melatonin and/or direct inhibition of the circadian drive for sleep emanating from the suprachiasmatic nuclei remains to be determined. Our current data did not demonstrate a strong positive association between EEG high-alpha power and melatonin suppression but did show a correlation between low melatonin levels and low delta-theta power. This latter finding indicates that the effects of increased homeostatic sleep pressure are weakened by the degree of melatonin suppression and that sleep pressure and melatonin may use a final common pathway in influencing delta-theta activity in the waking EEG. As these data must be considered preliminary given the limited dynamic range of responses to the single photon density and 2 wavelengths employed in this experiment, further investigation is warranted to test whether melatonin has a direct role in mediating alertness during the biological night and whether additional and/or redundant mechanisms exist that mediate the alerting effects of light during the biological day.

The differentiation observed in the wavelength dependence of the direct effects of light on EEG power density may have a neuroanatomic basis. For example, a complex network may exist with specific wavelengths preferentially stimulating different photoreceptor systems that project primarily to separate arousal systems. Altered spectral sensitivity for different nonvisual responses to light has been observed[29] and is indicated by the range of differentiation in projections from melanopsin-containing retinal ganglion cells to brain regions mediating such nonvisual responses.[42] Taken together, however, the present results indicate that the effects of short-wavelength light on EEG markers of alertness and arousal are mediated through interactions with both homeostatic and circadian processes.

The effects of light on the spectral composition of the waking EEG are of particular relevance for the understanding of how

light exposure affects neurobehavioral performance. Previously, it was shown that minute-by-minute fluctuations on an auditory detection performance task were correlated with concomitant changes in the waking EEG, with a reduction in auditory lapses associated with reduced power in the delta-theta range (4-5 Hz), and elevated power in the high-alpha (10-11 Hz) range in the Cz derivation.[52] We also showed that increased arousal, including improved auditory reaction time and reduced auditory lapses, was associated with reductions in delta-theta power and an increase in high-alpha power. Thus, the spontaneous minute-by-minute changes associated with improved auditory performance and the sustained changes induced by short-wavelength light both appear to arise from changes in brain mechanisms controlling central arousal, alertness, and performance.

We found no significant difference in cortisol levels during the 460-nm and 555-nm light exposures. The elevation of cortisol prior to monochromatic light exposure may have been due to subjects' anticipation of the extended novel experimental intervention[53] (Figure 4). This increase in general arousal of the hypothalamic-pituitary-adrenal axis, however, was not reflected in a parallel increase in performance. Light-induced elevation of cortisol has only been observed previously during morning light exposure and can vary with experimental conditions,[54] and, therefore, the effect may be time-of-day and setting dependent.[6,55,56] Future protocols to assess the wavelength sensitivity for light-induced elevation of cortisol will need to address such experimental confounds.

Our findings suggest that long-duration short-wavelength light exposure is an effective potential countermeasure for fatigue and performance decrements, particularly during the biological night. Routine tasks that require sustained vigilance are most likely to be enhanced by exposure to short-wavelength-enriched light, for example, prolonged driving or extended safety monitoring (e.g., air-traffic controllers, airport baggage inspectors). Given, however, that short-wavelength light is a highly effective wavelength for phase-shifting the circadian pacemaker, suppressing melatonin, and activating the autonomic nervous system,[26,28,29] utilization or avoidance of such light exposure requires careful consideration and must be incorporated appropriately into schedules to ensure that the other effects of light do not result in undesirable side effects. The blue shift in spectral sensitivity of these responses relative to the visual photopic system also means that standard light-measurement techniques and equipment that utilizee a photopic weighting for illuminance measures (lux) are inappropriate when calculating effective exposures for nonvisual effects of light. Architects and lighting engineers will need to evaluate both the visual and neurobiological impact of light exposure when designing the interaction between natural and artificial light exposure in work and home environments.

In summary, we have demonstrated that short-wavelength light is more effective at stimulating subjective and objective correlates of alertness and performance, similar to other effects of light such as circadian phase shifting and melatonin suppression. The results suggest that conventional visual photoreception is not the major mediator of these responses.

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