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Preliminary Evidence That Both Red and Blue Lights Increase Nocturnal Alertness

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ABSTRACT
Retinal blue light exposures impact nocturnal alertness, implicating participation by the circadian system, which is maximally sensitive to short-wavelength light. We investigated the impact of two levels of both blue and red light on nocturnal alertness, as measured by electroencephalogram (EEG). Exposures to both levels of the blue and red lights increased beta and reduced alpha power relative to preceding dim-light conditions. These results suggest that the circadian system is not the only light-sensitive pathway that can affect nocturnal alertness because the levels of red light used here are not effective for stimulating the circadian system.

Keywords
Circadian, alertness, light, electroencephalogram

BACKGROUND
The impact of light on alertness has gained recent attention in the scientific community because of the now well-established role that retinal light exposure plays in regulating extra-visual functions like the circadian timing system. Bright white light (greater than 2500 lx at the eye) has been shown to increase alertness at night [2, 3, 5, 7-10, 12, 17], but the mechanisms associated with the alerting effects of light have not been unambiguously established.

The human circadian system is known to be maximally sensitive to short-wavelength radiation (blue light) [4, 18, 25]; thus, the efficacy of "moderate" blue light should be comparable to that of "bright" white light for evoking measures of alertness at night [18]. Results from recent studies using blue light [6, 13] are entirely consistent with the neurophysiological evidence that neural pathways from the suprachiasmatic nuclei (SCN) affect sleep and alertness, as recently elucidated by Saper and colleagues [20-22], adding weight to the inference that the SCN, through retinal stimulation by short-wavelength light, play a role in human nocturnal alertness.

One way to test the hypothesis that the alerting effects of nocturnal light exposures are mediated only by the circadian system is to compare the impact of red and blue light on alertness at night. Long-wavelength ($\lambda > 600$ nm) light does not stimulate the human circadian system [4, 25] except perhaps at very high levels [14]. Therefore, if the light-induced stimulation of the circadian system at night is solely responsible for light-induced nocturnal alertness, then red light should be an ineffective stimulus.

The present experiment was designed to look at the impact that two "moderate" levels (10 lx and 40 lx) of both narrow-band blue (λ_{max} = 470 nm) and narrow-band red (λ_{max} = 630 nm) light might have on electroencephalogram (EEG) recordings during the night. Electrodes affixed to the human scalp are able to sense changes in brain activity when subjects engage in different types of mental tasks. The relative electrical power recorded at these different frequencies (from 1 to 50 Hz) is used to infer mental states in these subjects. Changes in the power at specific different frequency bands have been used as markers of mental alertness. Alertness is associated with lower levels of power in the alpha frequency band (8-12 Hz) and is associated with higher levels of power in the beta frequency band (12-30 Hz). If the circadian pathway is solely responsible for light-induced alertness at night, then only the blue light should reliably evoke an alerting response. Further, there should be a graded response in the EEG recordings with increasing levels of the blue light, as long as their irradiances are above threshold and below saturation for the circadian system response.

METHODS

Procedures and apparatus
Sixteen subjects (21 to 33 years of age) were recruited to participate in the study from an electronic posting at Rensselaer Polytechnic Institute in Troy, N.Y. All subjects were screened for major health problems and except for women taking birth control pills, subjects reported not taking any pharmaceuticals or medications. Every subject completed a Munich Chronotype Questionnaire (MCTQ) prior to the study [19]. In order to have a more homogenous sample of subjects, those who were late or extremely late chronotypes were excluded from the experiment. All subjects provided an informed consent approved by Rensselaer's Institute Review Board. Subjects were asked to refrain from alcohol and caffeine on the days of the experiment and were asked not to sleep after awakening for the day. Of the sixteen subjects, nine males and five females completed the entire experiment, and the results of their EEG data are reported here.

Four experimental lighting conditions, two spectra (blue and red) each at two light levels (10 and 40 lx) were deli-

vered to individual subjects from 0.6 × 0.6 × 0.6 m light boxes, each fitted with arrays of light-emitting diodes (LEDs). The arrays (ICove, Color Kinetics) were located behind the front box apertures to be outside the subject's direct view, thereby creating a uniform, non-glaring distribution of light within the box. During light exposures, subjects placed their chin on a chinrest mounted near the front of a box, ensuring delivery of the prescribed light exposure. When sitting at the light box, the subject's head was aligned with the aperture of the box, so that subjects were always exposed to full-field, diffuse light. The spectral emissions of the blue LEDs peaked at 470 nm with a full width at half maximum (FWHM) of 25 nm. The red LEDs peaked at 630 nm with a FWHM of 25 nm. Before the experiment, each of the light boxes was calibrated using a Gigahertz illuminance photometer to provide the prescribed corneal illuminance levels when the subjects were positioned on the chinrest. The spectral irradiance of the red and blue conditions were measured prior to the experiment with a calibrated spectroradiometer (Photoresearch model PR705a) and diffuse white reflectance standard (Labsphere model SR 099) and used to calibrate the Gigahertz illuminance readings. Two boxes provided blue light (40 $\mu W/cm^2$ at 40 lx and 10 $\mu W/cm^2$ at 10 lx) and two emitted red light (19 $\mu W/cm^2$ at 40 lx and 4.7 $\mu W/cm^2$ at 10 lx); light levels could be adjusted with an electronic dimmer to reach the prescribed light levels without significantly affecting the relative spectral distributions of the LED emissions. Measurements of pupil area completed after the experiment with a different group of subjects (N = 5) were: red at 10 lx, 34 mm^2; red at 40 lx, 22 mm^2; blue at 10 lx, 10 mm^2; blue at 40 lx, 6.5 mm^2.

Groups of four subjects participated in two sessions separated by at least one week. Subjects were asked to arrive at the laboratory at 22:00 to receive instructions and be fitted with scalp electrodes for EEG recordings. Because only one EEG machine was available, data collection was staggered. The first subject in a session started at 23:00, the second at 23:10, the third at 23:20, and the last at 23:30; the last subject completed the experiment at 03:45. During every session, each subject was presented a high (40 lx) and a low (10 lx) light exposure condition of the same spectrum (blue or red). The presentation order of the light levels was counterbalanced across sessions for a given subject; light spectra were counterbalanced across subjects within sessions. Every 45-minute experimental lighting condition was preceded by a 45-minute period of inactivity in a dim-light anteroom (< 1 lx of red light at the cornea). During the inactive, dim-light periods, subjects remained quiet and were not allowed to perform any task (e.g., talk, read, or computer work) except for the prescribed data sampling specified in the experimental protocol. Each nighttime session consisted of four, 45-minute light-and-dim conditions (a dim-light condition always preceded one of the four experimental lighting conditions), plus a 15-minute period for data collection prior to each lighting condition (in addition to EEG recording, performance measures and saliva melatonin were also collected, but are not reported here).

Data Collection

The Biosemi ActiveTwo system with active electrodes was used for EEG recordings. This system is battery powered, minimizing electrical interference from alternating current (ac) during recording sessions. Electrodes were placed on subjects' scalps according to the International 10-20 system at Oz, Pz, Cz, and Fz [1]. Two additional electrodes serving as virtual reference electrodes for those attached to the scalp were attached to the right and to the left earlobes.

Near the end of each 45-min dim light and each 45-min light exposure period, the scalp electrodes on each subject were attached to the EEG recording system. Six minutes of data were collected: three one-minute periods with the subject's eyes closed alternating with three one-minute periods with the eyes open. When the eyes were open and subjects were not sitting at the light box (dim-light condition), the subjects were asked to fixate on a specific marked point approximately one meter away. Similarly, when sitting at the light box, subjects fixated on specific point on the far wall of the box approximately 0.6 m away. Subjects were visually monitored by an experimenter to ensure compliance with the protocol.

The EEG signals were sampled at 16384 Hz and then low-pass filtered and downsampled to 2048 Hz for electronic storage by the Biosemi system. All subsequent EEG data processing and analyses were performed with Matlab version R2008a by The Mathworks™. The signals recorded from the two reference channels were averaged and these values were subtracted from those obtained from all of the other channels. The direct current (dc) offset of each channel was eliminated by subtracting the mean value of each channel from itself. A low-pass finite impulse response (FIR) filter ($f\text{-}_{3dB}$ = 50 Hz) was applied and the data were downsampled to 512 Hz. Then a high-pass, third-order Butterworth filter ($f\text{-}_{3dB}$ = 4 Hz) was applied to the downsampled signals from each channel to eliminate slow trending in the data.

Another program divided the filtered data into 5-second epochs, segregated by periods when the eyes were open and when they were closed during the six-minute recording period. Eye blink artifacts were eliminated by removing epochs from all channels where voltage fluctuations of any epoch exceeded ±100 μV. A Blackman window followed by a fast Fourier transform (FFT) was then applied to the data segments. This process yielded spectral power distributions from 1-50 Hz. The power spectra for each one-minute segment were then combined to give an average spectral power distribution for each trial. The relative power levels for eyes open in the alpha (8-12 Hz), beta (12-30 Hz), gamma (30-50 Hz), theta (5-8 Hz), and alpha-theta (5-9 Hz) ranges were calculated as a percentage of overall power from 1-50 Hz. These calculations were not performed for those intervals when the eyes were closed. Reported here are the results from the percentages of power

in the alpha and the beta range of frequencies because they have been used as measures of alertness in previous studies (e.g., [6]).

RESULTS

Two-way (eight light-and-dim conditions and four recording channels), repeated measures ANOVAs were employed using the percent power in the alpha frequency range (8-12 Hz) and using the percent power in the beta frequency range (12-30 Hz) recorded from four scalp electrode channels (Oz, Pz, Cz and Fz) in the EEG recordings. Both the main effects of light-and-dim conditions and of recording channels were significant for alpha (respectively, $F_{7,91}$ = 2.15, p = 0.046 and $F_{3,39}$ = 44.7, p <0.0001) and for beta (respectively, $F_{7,91}$ = 3.91, p = 0.0009 and $F_{3,39}$ = 5.36, p = 0.0035); the interaction between the light-and-dim conditions and the channels was not statistically significant for either the alpha or the beta frequencies, indicating that the alpha and the beta frequencies from every channel exhibited similar patterns among the eight light-and-dim lighting conditions. Post-hoc, paired two-tail t-tests were performed for the alpha and for the beta frequencies using the combined data from all four channels.

As illustrated in Figure 1, relative alpha power recorded from all channels after light exposure decreased compared to relative alpha power recorded in the dim light just prior to the light exposure. Alpha power after exposures to both levels of blue light (i.e., 10 lx and 40 lx) and to red light at 10 lx was statistically significantly lower than alpha power recorded in the previous dim-light condition. Consistent with Figure 1, Figure 2 illustrates the increase in relative beta power following light exposures compared to relative beta power recorded in the dim light just prior to the light exposures. Mirroring the statistical inferences for the alpha frequencies, there was a significant difference between the previous dim-light condition and the two blue-light conditions and for the red-light condition at 10 lx.

It was hypothesized that the blue light conditions would follow a dose response such that relative alpha power would be lower for the 40 lx condition than for the 10 lx condition. It was also expected that the relative beta power would be significantly higher for the blue-40 lx condition than for the blue-10 lx condition. This expectation was met for the alpha frequencies, (p = 0.01) but, although in the right direction, not for the beta frequencies (p = 0.1). Although the red-light condition resulted in dose intransitivity for both the alpha and the beta frequencies (i.e., the red-10 lx condition produced *lower* relative alpha power and *higher* relative beta power than for the red-40 lx condition), this difference was not statistically different for either frequency band (p = 0.21 for alpha power and p = 0.13 for beta power).

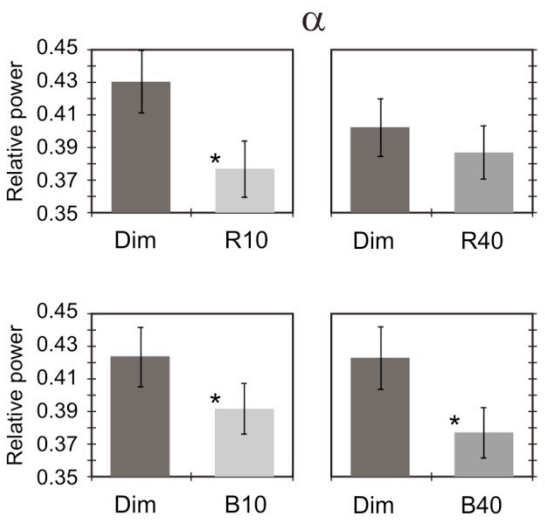

Figure 1. Relative alpha power after the four dim and the four experimental lighting conditions. Statistically significant (*) lower levels of relative alpha power were associated with blue-10 lx (B10; p = 0.007), blue-40 lx (B40; p<0.0001), and red-10 lx (R10; p< 0.0001), than with the previous dim-light exposures. There was no significant difference in alpha power between red-40 lx (R40) and the previous dim-light exposure which must, in part at least, reflect the significantly lower (p < 0.05) alpha power level associated with the dim condition preceding the R40 condition than with any of the other the dim condition.

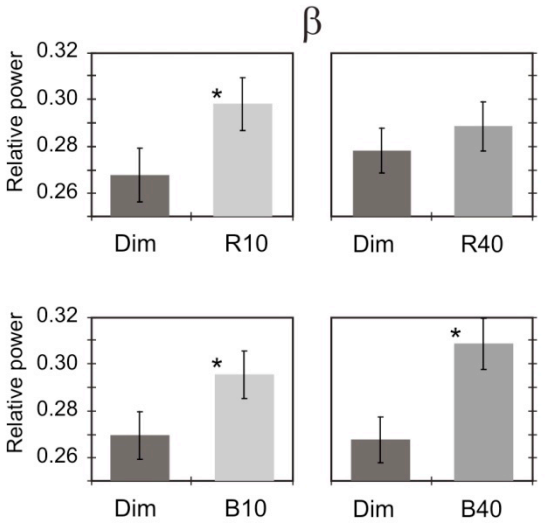

Figure 2. Relative beta power after the four dim and the four experimental lighting conditions. Statistically significant (*) higher levels of relative beta power were associated with blue-10 lx (B10; p = 0.0006), blue-40 lx (B40; p<0.0001), and red-10 lx (R10; p< 0.0001), than with the previous dim-light exposures. There was no significant difference in beta power between red-40 lx (R40) and the previous dim-light exposure.

DISCUSSION

Nocturnal alertness as measured by EEG is affected by light, but it does not seem to be affected only by light stimulation of the circadian system. Exposures to both red and blue light reduced alpha power and increased beta power levels relative to their preceding dim-light condition. However, there was only an apparent dose response for the blue light. That is, as levels of blue light increased from 10 lx to 40 lx, alpha power decreased and beta power increased (although not statistically significant), as would be expected if blue light served as alerting stimuli. Quizzically, the reverse was true for the red light. Although not significant, alpha levels were higher and beta levels were lower for 40 lx than for 10 lx of red light. If in fact reliable, the dose intransitivity for the red-light conditions remains unexplained and, indeed, somewhat implausible. It is conceivable that there is an optimum irradiance of red light for alertness (i.e., red-10 lx), but this inference seems rather unlikely and these results definitely demand further study. Nevertheless, these results indicate that colored light of ”moderate” corneal irradiance levels can induce alertness at night, but that light-induced alertness at night is not mediated only by the circadian system.

It is not completely clear, however, how light-induced alertness can arise from other neural pathways. Some evidence suggests that red light, which is ineffective for stimulating the circadian system at “low” and “moderate” light levels, can be more stimulating than blue light [15, 24]. Studies have reported that perception of red color prior to executing an important task impairs performance relative to the perception of green or achromatic color [11, 15]. Elliot et al. [11] performed a series of studies to investigate the impact of color red on performance in achievement contexts, that is, in situations in which competence is evaluated and positive and negative outcomes are possible. They hypothesized that red color is associated with danger of failure, and therefore, an automatic, unconscious decision to avoid the object, situation or event occurs. According to their hypothesis, red color impairs performance because it evokes motivational tendency to avoid failure, which, according to the authors, undermines performance. The results of their experiments supported their hypothesis that perception of red color prior to an achievement task impairs performance compared to a green and an achromatic color. Similar findings have been reported by Stone [23]. These results are not consistent with findings by Hill and Barton [15], however, who reported that red enhances performance of athletes who wore red color. In general, the studies of color on emotions and performance are conflicting and not well-grounded in neurophysiology. The explanation for this lack of consistency may be due to random, non-systematic effects of color on human perception or psychology or to individual differences in preference and cultural associations [16]. Notwithstanding this last point, these results are then, to a limited extent, consistent with some previous studies suggesting that red light acts as a stimulant through some unspecified neural pathway. It is important to note, however, that these earlier studies (e. g., [11, 15, 23, 24]) were probably conducted in the daytime, not at night, although the times for these studies were not documented. Further, these studies do not always provide quantitative descriptions of the colored stimulus. Clearly, more research is needed to elucidate the light-sensitive mechanisms affecting alertness during the day and during the night.

CONCLUSIONS

The present results are consistent with previous findings showing that light of sufficient corneal irradiance increases alertness at night. There is previous compelling evidence that light-induced stimulation of the circadian system increases alertness at night, but the present results implicate other mechanisms through which light can also increase alertness. It is important then to determine if these inferred mechanisms are independent of the circadian system or interact with it by conducting more systematic studies of light spectra and light levels during the night as well as during the day.

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