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The effects of red and blue light on alertness and mood at night

B Plitnick RN, **MG Figueiro** PhD, **B Wood** and **MS Rea** PhD FSLL
Lighting Research Center, Rensselaer Polytechnic Institute, 21 Union Street, Troy, NY 12180, USA

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This study was designed to explore the roles that long- and short-wavelength lights have on momentary mood and alertness at night. Twenty-two subjects participated in a mixed-design experiment, where we measured the impact of two levels of long- and short-wavelength lights on brain activity and on self-assessments of alertness, sleepiness and mood. Measurements were obtained 60 minutes prior to, during and after light exposure. Results showed that the red and the blue lights increased electroencephalographic beta power (12–30 Hz), reduced sleepiness, and increased positive affect relative to the previous dim-light period indicating that alertness and mood can be affected by light without necessarily stimulating the melatonin pathway. The impact of light was modest, however, compared to the increase in fatigue over the course of the night.

1. Background

Environmental stimuli can affect momentary mood and alertness. The impact of colour on momentary mood and alertness is still controversial, at least in part because of the confusion between three distinct domains, physical, physiological and psychological. Strictly speaking colour is not a stimulus, but rather a psychological result of processing by neural mechanisms of the spectral irradiance distribution (SID) incident on the retina. Some authors suggest that apparent colour itself affects momentary mood and alertness. Other studies have examined the impact of different SIDs on hormone production and electrical brain activity associated with momentary mood and alertness without specific regard to the apparent colour those stimuli evoke. It is usually very difficult, however, to determine the underlying mechanisms contributing to light-induced changes in momentary mood and alertness because optical radiation incident on the retina has multiple effects on brain activity through parallel neural pathways.

For example, some studies suggest that colours seen as 'warm' (red, orange, yellow) evoke feelings of arousal while colours seen as 'cool' (violet, blue, green) are associated with calming feelings. The colour red, for example, has been associated with feelings of danger, love, rage and excitement as well as negative feelings such as anxiety, anger and annoyance. It has been suggested that the colour red increases human receptiveness to external stimuli and increases excitation, therefore affecting a person's emotional and motor responses.[1,2] Goldstein has argued that red colours, though arousing, impair performance on complex activities in which exactness is required. The colours green and blue have been associated with feelings of relaxation and calmness.[2–6]

Elliot *et al.*[7] performed a series of studies to investigate the impact of red colour on performance in achievement contexts, that is, in situations in which competence is

Address for correspondence: MG Figueiro, Lighting Research Center, Rensselaer Polytechnic Institute, 21 Union Street, Troy, NY 12180, USA. E-mail: figuem@rpi.edu

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evaluated and positive and negative outcomes are possible. They hypothesised that red colour is associated with danger of failure, and therefore, there would be an automatic, unconscious decision to avoid the object, situation or events. Their findings supported the hypothesis that perception of red colour prior to an achievement task impairs performance compared to a green and an achromatic colour. Similar findings have been reported by Stone.[8] These results are not consistent with findings by Hill and Barton,[9] however, who reported that red enhances performance of athletes who wore red colour. In general, the studies of colour on emotions and performance are conflicting. Although part of the explanation for this lack of consistency may be due to random, non-systematic effects of apparent colour on human physiology and behavior, or to individual differences in apparent colour preference, or to differences in cultural associations with apparent colour,[10] some of the uncertainty must arise from a failure to define the light stimulus independent of the psychological response that the stimulus evokes, that is, its apparent colour. Without a characterisation of the stimulus independent of the response it evokes, it is not possible to develop an understanding of the physiological mechanisms mediating the measured outcome.

Other studies have examined the impact of SIDs (combinations of both spectrum and irradiance), not simply apparent colour, on alertness, sleepiness and performance.[11,12] Of particular interest for the present study, several studies have examined the impact of short-wavelength light on nocturnal alertness and performance as it might be mediated by the suprachiasmatic nuclei (SCN), the pacemaker for regulating circadian rhythms. Short-wavelength light is an important stimulus to the human circadian system; it is known to be maximally sensitive to short-wavelength radiation peaking at about 450 nm. Presumably, it is only a collateral effect that short-wavelength light is also seen as blue in these studies. This assumption has justification because the results of recent studies using short-wavelength light as a stimulus for enhancing alertness and performance and reducing sleepiness are entirely consistent with the neurophysiological evidence that neural pathways from the SCN are important to sleep and alertness.[13] These converging lines of research add weight to the inference that the SCN, through retinal stimulation by short-wavelength light, plays an important role in human alertness and probably performance. Very recently, however, Figueiro and colleagues[14] showed that the circadian system may not be the only pathway associated with light-induced alertness at night. They showed that both long-wavelength, red light and short-wavelength, blue light of the same corneal illuminance evoked similar alerting effects (i.e. increased power in the electroencephalogram (EEG) beta frequencies and reduced power in the EEG alpha frequencies).

The goal of this study was to expand on that work by Figueiro and colleagues by investigating the impact of long-wavelength, red and short-wavelength, blue light on measures of sleepiness and momentary mood (both measured subjectively). The present study was expected to serve as a partial validation of the previous results as well as to investigate the dissipation of the alerting effects of light after exposures to blue and red lights.

2. Methodology

In order to minimise differences in cultural biases toward colours, 24 native-born subjects from the United States (19–27 years of age) were recruited to participate in the study from an electronic posting at Rensselaer Polytechnic Institute in Troy, New York. 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; those who were late or extremely late chronotypes were excluded from the experiment. This study was approved by the Rensselaer Polytechnic Institute Institutional 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. Subjects were also asked to go to bed no later than 23:00 the night before the experiment. Of the 24 individuals recruited for the study, 22 (9 males and 13 females) subjects completed the entire experiment and their results are reported here. Two subjects decided to withdraw from the experiment after the first session.

Four experimental lighting conditions, two spectra (blue and red) at two levels (10 lx and 40 lx), were delivered to individual subjects from $0.6 \times 0.6 \times 0.6\,m^3$ light boxes, each fitted with arrays of light-emitting diodes (LEDs). The arrays (ICove, Colour 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 rest mounted near the front of a box, ensuring delivery of the prescribed light exposure. 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 10 lx or 40 lx for both LED arrays at the plane of the subject's corneas when positioned in the chinrest. Two boxes provided blue light ($40.2\,\mu W/cm^2$ at 40 lx and $10\,\mu W/cm^2$ at 10 lx) and two emitted red light ($18.9\,\mu W/cm^2$ at 40 lx and $4.7\,\mu W/cm^2$ at 10 lx); light levels were adjusted with an electronic dimmer without significantly affecting the spectral irradiance distributions of the LEDs. 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, $6.5\,mm^2$.

The Biosemi ActiveTwo system with active electrodes was used for EEG recordings. This system is battery powered, minimising 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.[15] Two additional electrodes serving as virtual reference electrodes for those attached to the scalp were attached to the right and to the left earlobes. Another electrode was placed approximately 5 cm below the left clavicle to measure an electrocardiogram (ECG) signal.

To minimise introducing personal biases associated with colour into the experiment, a mixed-design experiment was conducted whereby half the subjects only saw blue light and half the subjects only saw red light. Subjects were given the same instructions before starting the experiment during which they were informed that the light colour they would be seeing (red or blue) was known to have an alerting effect and that we were investigating the impact of light level. The light stimuli were approximately equated in terms of visual response (i.e. illuminance at the cornea), not in terms of circadian response because equal circadian responses by both spectra would have resulted in exceedingly bright red-light conditions relative to the blue-light conditions. Marked disparities in the apparent brightness of the blue and red lights would have confounded interpretation of any subjective responses to the light stimuli because it would have been impossible to know whether subjects were responding differentially to the apparent brightness or to the apparent colour. The mixed-design study was conducted over the course of several weeks

during February and March 2009. Each subject saw one light level per night in a counterbalanced order. Twenty-four subjects were recruited originally, but since only 11 subjects in each group completed the study the planned counterbalancing was not complete; six subjects saw the blue and the red lights at 10 lx first while five subjects saw the blue and red lights at 40 lx first. All subjects were asked to arrive at the laboratory at 23:00 to receive instructions and be fitted with electrodes for EEG/ECG recordings. Groups of four subjects participated in two sessions separated by at least 1 week.

Every session began at 00:00 and was completed at 03:30. Figure 1 shows the experimental design for one subject. The start of data collection for each of the other three subjects in a session was successively staggered by 5 minutes. The 60-minute light exposure condition was preceded by a 60-minute period in dim light (<1 lx of red light ($\lambda_{max} = 630$ nm) at the cornea). Starting at midnight, subjects remained in the dim light for 60 minutes. Recordings of the first subjective assessments (sleepiness, alertness and momentary mood) in the dim light (D1) were completed after 15 minutes into the dim-light period. Self-assessments of sleepiness and alertness were performed using the Karolinska Sleepiness Scale (KSS)[16] and a modified Norris mood scale.[12,17] Self-assessments of momentary mood were obtained using the Positive and Negative Assessment Scale (PANAS).[18] KSS is a 9-point standardised sleepiness scale ranging from 'extremely alert' (1) to 'very sleepy, fighting sleep' (9). The modified Norris mood scale was comprised of 12 items; however, we only used responses to one, 7-point scale (−3, drowsy, to +3, alert) in the analyses. The PANAS scores are each based on 10, 5-point scales developed to independently measure positive affect and negative affect.

As shown in Figure 1, the first set of EEG/ECG measurements, at time (E)R, was obtained after 60 minutes in dim light, followed by another set of subjective assessments, at time (S)R, also collected in the dim light. Subjects were then asked to sit in front of the (red or blue) illuminated light box for 60 minutes. Subjective assessments (La) were

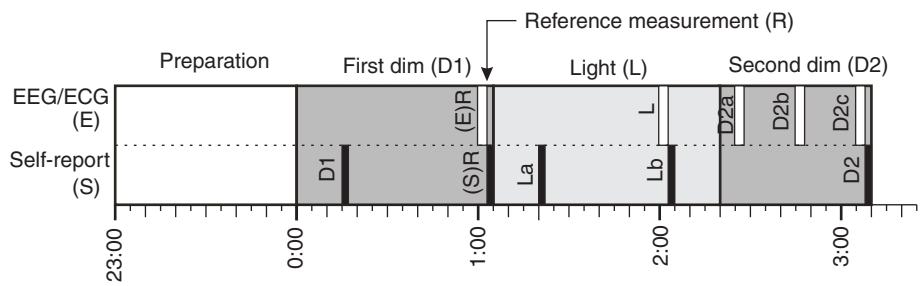

Figure 1 Four subjects were scheduled to arrive at the laboratory at 23:00 for preparation for an experimental session. Data collection for the four subjects in a session was staggered to permit sequential EEG/ECG recordings. Every session began at 00:00 and data collection was completed for the last of the four subjects at 03:30. Illustrated is the measurement time sequence for the first subject in a session. During every session, each subject was presented a high (40 lx) or a low (10 lx) light exposure condition of the same spectrum (blue or red). Subjects within a group received the same colour of light (red or blue), but half saw the lower and half the higher light level. EEG/ECG collection times (white bars): The reference measurement (E)R = after 60 minutes in the dim light, L = after 60 minutes in the light, D2a = 5 minutes after light was turned off; D2b = 25 minutes after light was turned off; D2c = 45 minutes after light was turned off. Self-report collection times (black bars): D1 = after 15 minutes in the first dim light condition, (S)R = after approximately 63 minutes in the dim light; La = after 15 minutes in the light condition, Lb = after approximately 63 minutes in the light condition; D2 = approximately 48 minutes after the light condition was completed

obtained 15 minutes after the red or blue light was turned on. After 60 minutes of light exposure, EEG/ECG measurements (L) and subjective assessment (Lb) were obtained again. Lights were turned off 20 minutes after data collection and subjects sat again in dim light. EEG/ECG measurements (D2a to D2c) were taken at 5, 25 and 45 minutes after the experimental treatment light was turned off. Subjective assessments (D2) were taken again after the final EEG/ECG measurements (45 minutes after the light was turned off). Subjects were asked to perform word searches or crossword puzzles continuously during the dim and light periods; these diversions were placed on clipboards so that subjects could keep their chins in the chin-rest for the prescribed exposure duration. For the last 15 minutes prior to EEG/ECG collection, subjects were asked to complete Sudoku puzzles. During the last 45-minute of data collection (after lights were turned off), subjects continuously worked on completing Sudoku puzzles until each collection time (5, 25 and 45 minutes after the lights were turned off).

Near the end of every dim and every light exposure period, the electrodes affixed to each subject in a session were, in turn, plugged into the recording system for EEG and ECG measurements. Three minutes of continuous data were collected from each subject. The subjects were asked to fixate on a specific marked point on the far side of the light box, approximately 1 m away. When in the dim light, subjects were asked to continue to sit in front of the light box, but the LEDs were not energised. Subjects were visually monitored by an experimenter to ensure compliance with the protocol.

3. Results

3.1 Self-report

Since the order of light level presentations were counterbalanced within groups across the two sessions, it was possible to statistically compare self-reports of alertness, sleepiness, and affect, both negative and positive, independent of the order in which the light levels were presented to the subjects.

To minimise inherent idiosyncrasies among the subjects, the difference between responses given by the subjects during the reference measurement time (S)R, after 60 minutes in the dim light, and responses given during the other conditions (D1, La, Lb and D2) were used to evaluate the experimental conditions. Thus, difference scores on the Norris scale, the KSS scale and the PANAS Positive and Negative scales were each submitted to a one-between (colour) by two-within (light level and time) mixed-design analysis of variance (ANOVA). Responses to questions on the Norris scale and on the PANAS Negative scale exhibited no statistically significant effects and are not discussed further.

Difference scores for the KSS and the PANAS Positive scales were quite similar, both showing a significant main effect of time ($F_{3,60} = 18.3$, $p < 0.0001$ for KSS and $F_{3,60} = 39.5$, $p < 0.0001$ for PANAS Positive). A significant light level × time × colour interaction ($F_{3,60} = 7.3$, $p = 0.0003$) was found for KSS difference scores and for the PANAS Positive difference scores ($F_{3,60} = 2.83$, $p = 0.046$). For the PANAS Positive scale, student's two tail *t*-tests were performed for the combined light level data and revealed a significant difference between (S)R-D1 and (S)R-La ($p < 0.0001$), (S)R-D1 and (S)R-Lb ($p < 0.0001$), (S)R-D1 and (S)R-D2 ($p < 0.0001$). For the KSS, significant differences between (S)R-D1 and (S)R-La ($p < 0.0001$), (S)R-D1 and (S)R-Lb ($p < 0.0001$), (S)R-D1 and (S)R-D2 ($p < 0.0001$), (S)R-La and (S)R-D2 ($p = 0.005$) and (S)R-La and (S)R-Lb ($p = 0.002$) were obtained. To correct for multiple comparisons, the criterion alpha level (i.e. $p < 0.05$) was adjusted in accordance with the Bonferroni/Dunn method to

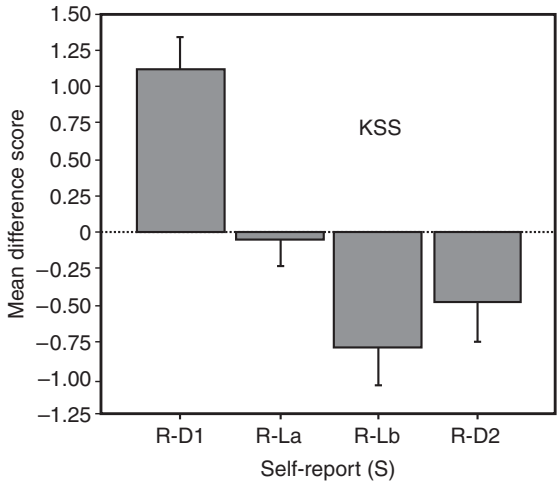

Figure 2 Mean difference scores ± SEM for responses to the KSS scale over the course of the night. Data for each subject are based upon the change in response at the reference time (S)R, that is, at the end of first dim-light period, to those at each of the other sampling periods D1, La, Lb and D2

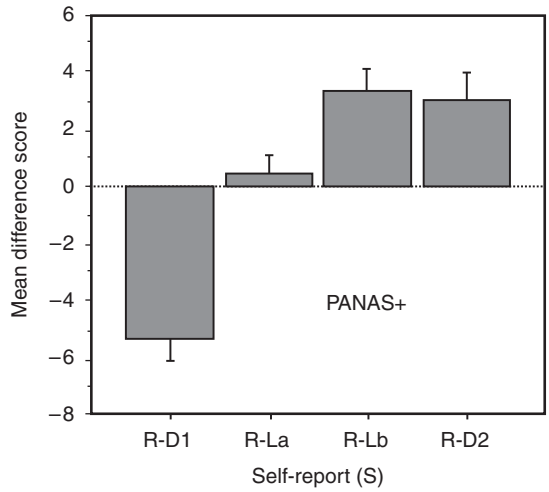

Figure 3 Mean difference scores ± SEM for responses to the PANAS Positive (+) scale over the course of the night. Data for each subject are based upon the change in response at the reference time (S)R, that is, at the end of first dim-light period, to those at each of the other sampling periods, D1, La, Lb and D2

$p < 0.0083$. Figure 2 shows the mean difference scores ± standard error of the mean (SEM) for the KSS difference scores and Figure 3 shows the mean difference scores and SEM for the PANAS Positive difference scores. These figures also show that the mean difference scores for the KSS and the PANAS Positive scales are effectively mirror images of one another. Whether this close correspondence between the two self-report scales has fundamental significance (i.e. increased sleepiness leads to reduced positive affect but not increased negative affect) cannot be determined from this experiment. A future study, specifically aimed at determining if such relationships exist, would have to be conducted where attention was given to 'calibrating' the self-report data with objective measures of sleepiness and mood.[19]

Moreover, both red and blue lights had similar impact on KSS and PANAS Positive difference score, suggesting that there was no differential impact of light spectra on both measures. As the significant three-way interactions suggests, however, there were differential effects for the blue and red lights at 10 lx and 40 lx with the KSS and PANAS Positive difference scores or, perhaps, that subjects within the two groups associated with the blue and red lights responded differently on these self-report scales. The blue light at 40 lx had, as might be expected, a more positive effect on sleepiness and momentary mood than did the blue light at 10 lx. A different pattern resulted from the red light exposures. Red light at 10 lx had a greater impact on reducing sleepiness and improving positive affect than did the red light at 40 lx. Another experiment would need to be conducted to determine if this effect is reliable and, if so, what was its basis.

There was no evidence for persistence in these effects in the following dim-light periods using any of the self-report measures. Rather, there was only evidence for a steady increase in subjective sleepiness and reduction in positive affect over the course of the experiment using these scales.

3.2 EEG

The EEG signals were sampled at 16 384 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 ($f_{-3dB} = 50$ Hz) was applied and the data were downsampled to 512 Hz. Then a high-pass, third-order Butterworth filter ($f_{-3dB} = 4$ 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. Eye blink artefacts were eliminated by removing epochs from all channels where voltage fluctuations of any epoch exceeded $\pm 100\,\mu$V. 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 to 50 Hz. The power spectra for each 1-minute segment were then combined to give an average spectral power distribution for each trial. The relative power levels for the 3 minutes in the alpha (8–12 Hz), alpha–theta (5–9 Hz), theta (5–7 Hz), beta (12–30 Hz) and gamma (30–50 Hz) ranges were calculated as a percentage of overall power from 1 to 50 Hz.

The ECG data were digitally processed the same way as the EEG data up to the high-pass filtering. For the ECG analysis, the high-pass filtering −3dB cut-off was lowered to 0.2 Hz. Heart rates corresponding to the filtered ECG data were determined by two methods: (1) by taking the FFT of the ECG, whereby the frequency having the peak power within the range from 40 to 120 beats/minute is the heart rate, and (2) determining the elapsed time between the QRS complexes (the successive peaks, Q then R then S, in the ECG signal) of the ECG.[20] The QRS complex represents ventricular depolarisation. It is called a complex because there are three different waves in it (Q-wave, R-wave and S-wave). The QRS complexes were located by the first derivative of the ECG falling below a negative threshold value after individual normalisation of first derivative of the ECG.

As with the self-report data, the differences between relative power in the alpha and the beta frequency bands obtained from each subject after 60 minutes in the dim light (E)R and during the subsequent conditions (L, D2a, D2b and D2c) were used to evaluate the experimental conditions. Power differences in the alpha and in the beta frequency bands were both submitted to a one-between (colour) by three-within (light level, time of measurement and channel) mixed-design ANOVA. A statistical significant main effect of time of measurement was found for the power differences in the beta frequencies ($F_{3,60} = 4.0$, $p = 0.012$). Figure 4 shows that unlike the positive change in the relative power in the beta frequencies for subsequent dim-light conditions, the change in relative power in the beta frequencies was negative for the light exposure condition, (E)R-L, indicating higher relative beta power during the light exposure period than during the preceding dim-light period. *Post hoc* two-tail student's *t*-tests on the beta power differences were performed and revealed a significant difference between (E)R-L and (E)R-D2a ($p = 0.046$), (E)R-L and (E)R-D2b ($p = 0.015$) and (E)R-L and (E)R-D2c ($p = 0.0016$). It is perhaps worth noting too, that the gradual increase in the difference between beta power after the light exposure (L) and after dim-light exposures (D2a, D2b and D2c) suggests a gradual dissipation of the light's alerting effect; the difference after 5 minutes is smaller than after 45 minutes in dim light. Consistent with the

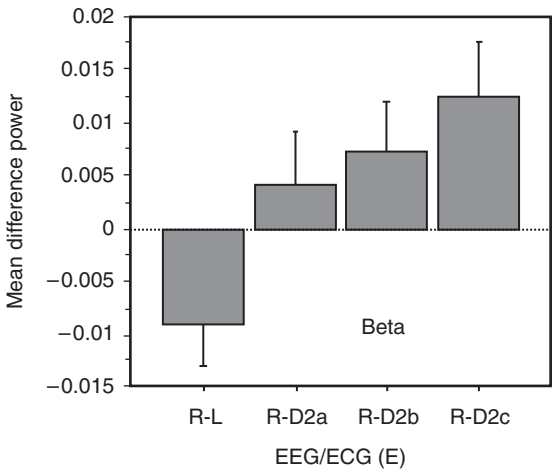

Figure 4 Mean difference and SEM for beta power. Data for each subject are based upon the change in relative power in the beta frequency range from the end of the first dim-light period, (E)R, to those at each of the other sampling periods, L, D2a, D2b and D2c

self-report data, beta power after exposure to both red and blue lights increased relative to beta power after the previous dim-light period, suggesting that both light spectra impacted brain activities.

These results are consistent with the hypothesis that mental activity increased in the light exposure conditions relative to the dim conditions. Consistent with the results for changes in beta power, although they were not statistically significant, the change in relative power in the alpha frequency band was greatest for the subsequent light exposure period, with progressively smaller differences associated with each successive dim period.

3.3 Heart rate

The FFT and QRS heart rate data were treated in the same way as the self-report and EEG data. The differences between heart rates measured after 60 minutes in the dim period, (E)R, and during the subsequent conditions (L, D2a, D2b and D2c) were submitted to a one-between (colour) by two-within (light level and time) mixed-design ANOVA. No significant effects were obtained using the FFT method but there was a significant colour by time of measurement interaction ($F_{3,60} = 3.2$, $p = 0.03$) using the QRS method. The difference between (E)R and L for blue light conditions was significantly greater ($p = 0.02$) than for the red light conditions. This interaction is difficult to interpret because the effect may be due either to differential effects of the two light spectra on heart rate or to differential responses to light by the two groups of subjects who each saw different spectra. A different experimental design would be needed to resolve the question.

4. Discussion

The present study investigated the impact of long-wavelength, red and short-wavelength, blue light on subjective and objective measurements of alertness, sleepiness and momentary mood. Although the mixed-design experiment did not allow for a precise comparison of effects of the two light spectra, both the red and the blue lights reduced self-reports of sleepiness (decreased KSS) and improved self-reports of momentary mood (increased positive affect on the PANAS scale). Objectively, the light stimuli increased alertness relative to the dim condition as measured by the power in the beta frequency range of the EEG recordings. There was also a gradual reduction in beta power after the blue and red lights were turned off suggesting a small persistence effect of light on alertness. Further studies are recommended to investigate the persistence of light's effect after the stimulus is removed.

In general, these results are consistent with the literature in that light can have an influence on people at night as measured by increased brain activity, reduced self-reports of sleepiness, and improved self-reports of momentary mood. It is still not clear, however, how light stimuli affect these outcomes.

It seems well established now that light, acting through the SCN, can affect sleep and alertness at night. Consistent with previous studies,[14,21,22] however, it would also seem that more than one mechanism, not just the melatonin pathway, must be involved because both long-wavelength, red and short-wavelength, blue lights led to similar effects in the present experiment. Finally, it should be pointed out that developing an understanding of these mechanisms will not be simple because the effects of light on alertness and momentary mood are relatively small compared to the overall increase in fatigue experienced by subjects throughout the long periods of wakefulness required during the study nights.

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