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Differential effects of light wavelength in phase advancing the melatonin rhythm

Abstract: Shorter wavelength light has been shown to be more effective than longer wavelengths in suppressing nocturnal melatonin and phase delaying the melatonin rhythm. In the present study, different wavelengths of light were evaluated for their capacity to phase advance the saliva melatonin rhythm. Two long wavelengths, 595 nm (amber) and 660 nm (red) and three shorter wavelengths, 470 nm (blue), 497 nm (blue/green), and 525 nm (green) were compared with a no-light control condition. Light was administered via a portable light source comprising two light-emitting diodes per eye, with the irradiance of each diode set at 65 $\mu W/cm^2$. Forty-two volunteers participated in up to six conditions resulting in 15 per condition. For the active light conditions, a 2-hr light pulse was administered from 06:00 hr on two consecutive mornings. Half-hourly saliva samples were collected on the evening prior to the first light pulse and the evening following the second light pulse. The time of melatonin onset was calculated for each night and the difference was calculated as a measure of phase advance. The shorter wavelengths of 470, 495 and 525 nm showed the greatest melatonin onset advances ranging from approximately 40–65 min while the longer wavelengths produced no significant phase advance. These results strengthen earlier findings that the human circadian system is more sensitive to the short wavelengths of light than the longer wavelengths.

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Introduction

Early studies have shown that broad-spectrum white light can suppress melatonin and phase shift the melatonin and core body temperature rhythms in humans [1–3]. However, there is some support for the differential sensitivity of the circadian system and pineal activity to different wavelengths of light. Animal studies have demonstrated that melatonin suppression is more sensitive to shorter wavelength light than longer wavelengths [4–8]. In the original human study, when a small number of volunteers were exposed to equal photon density of five different wavelengths of monochromatic light, plasma melatonin was suppressed more effectively by light with a peak wavelength at 509 nm [9, 10]. More recently two extensive studies have demonstrated that light with a peak wavelength between 430 and 500 nm was more effective in suppressing nocturnal melatonin than the longer wavelengths [11, 12].

However, rather than melatonin suppression per se, it is the ability to change the phase of circadian rhythms which is most important for the treatment of circadian rhythm disorders. It has been suggested that there may be a different mechanism underlying the melatonin suppression signal and the phase shifting signal to the pineal gland [13]. It has been shown in rats that the suppression of melatonin by propanolol does not induce a phase change in the onset of 6-sulfatoxymleatonin [14]. In addition, a 5-HT_{2c} antagonist attenuated the acute suppression of melatonin production in rats following a light pulse, without affecting the subsequent phase delay in the onset of melatonin production [15]. Some human studies have failed to find significant correlations between the degree of melatonin suppression and the amount of phase change [16–18]. If there are perhaps different mechanisms that underlie melatonin suppression and phase shifting, then it would be important to directly test efficacy of different wavelengths of light in phase shifting the melatonin rhythm.

In a previous study, we found that shorter wavelength light not only induced greater melatonin suppression but also produced a greater phase delay of the melatonin rhythm than longer wavelengths [19]. Monochromatic light was administered via a portable light device comprising two light-emitting diodes (LEDs) per eye, producing 130 $\mu W/cm^2$ irradiance of light to each eye. A 2-hr light pulse was administered from midnight on a single occasion and saliva melatonin onset was measured before and on the night following the light pulse. The results showed that light with peaks at wavelengths of 470 nm (blue), 497 nm (blue/green) and 525 nm (green) were more effective in producing a phase delay of the melatonin rhythm (27–38 min) than a no-light control condition or light with peaks at longer wavelengths of 595 nm (amber) and 660 nm (red).

However, in attempting to phase advance circadian rhythms Zeitzer and colleagues [18] found that exposure

to red light on three consecutive mornings for 5 hr induced a phase advance of the melatonin and temperature rhythm of almost 1 hr. This raised the interesting possibility that there may be different photic mechanisms for phase advance and phase delay. It has been proposed that the mammalian suprachiasmatic nucleus may have clock genes that react differently to a morning or evening light pulse [20, 21]. Therefore it may be possible that the human circadian clock is sensitive to different wavelengths in the morning as compared with evening.

Very recently, Warman and colleagues [22] found that blue light alone could phase advance the circadian system. A single 4 hr light pulse (07:15–11:15 hr) of only moderately intense (28 $\mu W/cm^2$) short wavelength light (436 and 456 nm peaks) phase advanced melatonin and core body temperature phase markers from 20 to 51 min. This amount of phase advance was comparable with that produced in their study by intense (4300 $\mu W/cm^2$) white light which presumably would have contained considerably more light from longer wavelengths. This study would suggest that shorter wavelengths may be more effective in phase advance than long wavelength light. However, neither of these two previously mentioned phase advance studies [18, 22] compared different wavelengths of equal irradiance. Therefore, the aim of the present study was to compare the different wavelengths of light for effectiveness in phase advancing the saliva melatonin rhythm following morning light stimulation.

Material and methods

Subjects

Forty-two volunteers (29 females, 13 males) with a mean age of 27.6 yr (S.D. $\pm$ 9.1 yr) participated in as few as one and up to six conditions. Twelve participants were students completing a university course and 30 were paid volunteers. They were all in reported good health and were not habitual users of alcohol, caffeine or hypnotic medication. They were tested with the Ishihara test for color blindness and all had normal color vision. Participants completed a 7-day sleep/wake diary and had a mean sleep onset time at 23:00 hr and a mean wake up time at 07:22 hr. No participants were definite morning or evening types [23]. For the five participants who completed all six conditions, the order of the conditions was counterbalanced across subjects. The other participants were randomly assigned to conditions bringing the number of participants in each condition to 15. An ANOVA found no significant differences in age and sleep onset times between conditions. The study was conducted during autumn and winter, with average sunrise at 07:13 hr and sunset at 17:22 hr.

Light-emitting diodes

The portable light device comprised LEDs mounted on spectacle frames. The lenses were removed from spectacle frames and two LEDs per eye were mounted on metal leads attached to the lower rim of the frame for each eye. The light from each LED was directed at the center of the pupil of each eye at a distance of 12 mm from the corneal surface. Each LED was covered with a polycarbonate transparent sheath stretched over and secured at the base of each LED to provide greater diffusion of the light and greater homogeneity of the spread of light intensity across the front surface of the LEDs. Each diode was connected to a 9-V battery constant current power source using a thin, flexible power cable.

Each pair of LEDs was mounted approximately in the middle of the field of vision of each eye. During the light pulse period participants were instructed to view the television just above the visual field area stimulated by the LEDs. As it is impossible to visually accommodate an object at 12 mm distance, the LED produced a bright, unfocused disk of light subtending 20° angle of visual field. Thus the two LEDs per eye occupied two 20° diameter disks of bright light just below the central or macular area with normal viewing of the television. As the LEDs illuminated approximately the iris of the eye, small deviations of the direction of gaze in the order of 30° from the television set would not result in a decrease in effective illumination of the pupil.

The wavelengths compared were 660 (red), 595 (amber), 525 (green), 497 (blue/green), and 470 nm (blue), with approximate half-peak bandwidths ranging between 10 and 18 nm [19]. They were compared with a no-light control condition. The electrical input current was adjusted so that all LEDs were equated for irradiance value of 65 $\mu W/cm^2$. Therefore, each eye, irradiated with two LEDs, received 130 $\mu W/cm^2$ at the corneal surface.

Melatonin assay

Saliva melatonin was assessed from saliva samples collected by gently chewing on polyester swab salivettes for 2 min (Sarstedt, Numbrecht, Germany). Samples were stored frozen until delivery to the Department of Obstetrics and Gynaecology, The University of Adelaide. After thawing and centrifugation, saliva melatonin was assayed in duplicate using direct radioimmunoassay of 200 μL saliva using standards and reagents provided by Buhlmann Laboratories (Allschwil, Switzerland) [24]. This assay uses the G280 antibody developed in this laboratory and [125I]2-iodomelatonin as the radiogland. All samples from an individual were run in the same assay. The intra- and inter-assay coefficients of variation were <10 and $<14\%$ across the range of the standard curve.

Procedure

For the participants who completed more than one condition, there was at least a week between each light condition to allow circadian rhythms and sleep to return to normal. Each condition was conducted over three consecutive nights, starting at 18:00 hr on nights 1 and 3 and 20:00 hr on night 2. Participants completed their evening meal by 18:00 hr and avoided alcohol and caffeine-containing substances during the meal. After 18:00 hr they were asked to drink water only. From 18:00 hr room illumination was kept below 25 lux. Participants remained seated watching television or videos and only had visits to the toilet immediately after a half-hourly saliva collection

on nights 1 and 3. The television monitor did not contribute more than 20 lux to the illuminance at the level of the participants' eyes.

On the first night, participants collected saliva samples half-hourly from 19:00 to 24:00 hr. They then slept until 06:00 hr. On this first morning, from 06:00 to 08:00 hr they wore the LED glasses. For the control condition participants wore sunglasses for 2 hr from 06:00 hr to ensure low ambient morning light. From 08:00 hr participants continued with normal daytime activities and normal ambient light exposure. On night 2, participants were instructed to remain in dim light from 20:00 hr and could retire to bed at any time. On the second morning, participants again wore the LED glasses or sunglasses for 2 hr from 06:00 hr. Following the light pulse, participants went about their normal activities until 18:00 hr. From 18:00 hr on night 3, the same procedure was followed as night 1, but saliva collection began at 18:30 hr and was completed at 23:30 hr. Participants then retired to bed.

The time of dim light melatonin onset (DLMO) was determined as the time at which saliva melatonin concentration consistently exceeded two standard deviations above the mean of baseline values [24]. Using this criterion the average number of data points comprising the baseline for determination of the DLMO was 4.4. If the participants showed a marked and consistent increase in melatonin from the second or third data point this was taken as the melatonin onset. This occurred on 38% of DLMO determinations. A difference measure for melatonin onset advance was calculated (melatonin onset night 1 − melatonin onset night 3) for participants in each condition.

Statistics

One-way non-repeated ANOVA were used to investigate any differences in DLMO on night 1 and the amount of phase advance between the conditions.

Results

Melatonin concentration values for night 1 ranged from < 4 to 16 pM at 19:00 hr to a range of 57–551 pM at 24:00 hr. For the purpose of illustration, examples from three conditions for one participant are shown in Fig. 1A–C. The melatonin concentrations (pM) are shown for the no-light control condition, 660 nm (red) condition, and 470 nm blue condition for night 1 (solid line) and night 3 (dotted line) following two 2-hourly pulses of morning light stimulation. As can be seen from the graphs, for the control and 660 nm (red) conditions, there is little difference between the slope and timing of the night 1 and night 3 melatonin curve and DLMOs indicating no phase advance of the melatonin onset. However, it can be seen that for the shorter wavelength of 470 nm (blue) there is a clear phase advance of the melatonin curve and DLMO on night 3.

Comparison of baseline melatonin onset between the light conditions showed no significant main effect, $F_{5,84} = 1$, $P = 0.4$. Therefore, the time of melatonin onset on night 1, before light stimulation, was similar across conditions.

Fig. 2 shows the mean phase advance (min) for each condition. Phase advances were significantly different

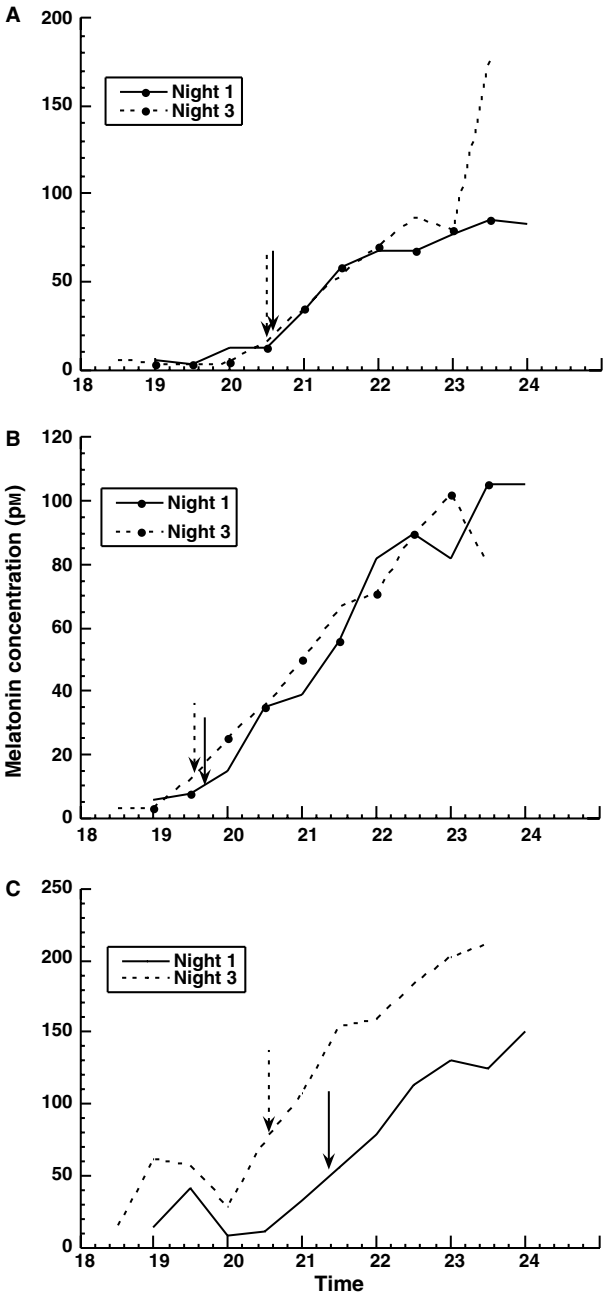

Fig. 1. Saliva melatonin concentrations (pM) of one participant on night 1 (solid line) and night 3 (dotted line) for the control condition (A), 660 nm (red) (B), and 470 nm (blue) (C) wavelength LEDs. Down arrows indicate the DLMO in each curve as determined by the formula in the Methods (solid arrow for night 1, dotted arrow for night 3).

between the different wavelength conditions $F_{5,84} = 7.9$, $P < 0.0001$. Post-hoc comparisons showed significantly more phase advance after morning light exposures of the shorter wavelengths of 470 nm (blue), 497 nm (blue/green) ($0 < 0.001$), and 525 nm (green) ($P = 0.006$) than the control condition. The shorter wavelengths of 497 and 470 nm also induced significantly more phase advance than the longer wavelength 595 and 660 nm light ($P < 0.005$).

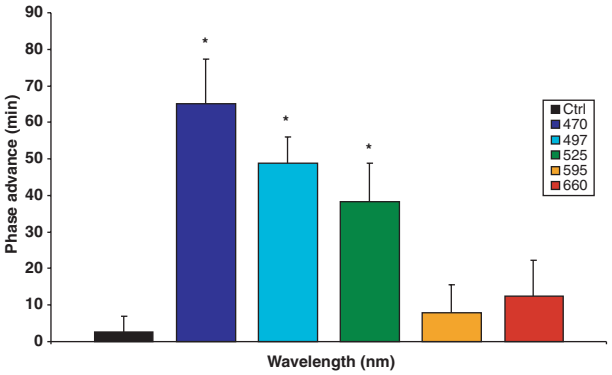

Fig. 2. Mean phase advance (min) (±S.E.M.) for the no-light control condition and each light condition. '*' Indicates significantly different ($P < 0.05$) compared with the control no-light condition and longer wavelength amber (595 nm) and red (660 nm) LED conditions.

The phase advance from the 525 nm was significantly greater than 595 nm ($P < 0.02$) and marginally greater than 660 nm ($P = 0.04$). There were no significant differences between the longer wavelengths and the control condition. Within the group of shorter wavelengths the 470 nm light produced a marginally greater phase advance than 525 nm ($P = 0.039$).

Discussion

The present study compared the effectiveness of the five different wavelengths of light and a no-light control condition in phase advancing the melatonin rhythm. After 2 hr of light stimulation on two consecutive mornings, the blue LED with a peak at 470 nm wavelength phase advanced the melatonin onset by over 1 hr. Similarly, the blue/green (497 nm) and green (525 nm) LEDs induced phase advances of almost 50 and 40 min, respectively. These phase advances were significantly greater than those of the control, no-light condition, as well as the longer wavelengths.

The present data, with no significant phase advance from the long wavelengths of 595 and 660 nm ($P > 0.40$), were not consistent with the results of Zietzer and colleagues [18] who found phase advances of the melatonin and core body temperature rhythms following morning exposure to red light. However, their use of 15 hr (3 × 5 hr) of red light at 200 lux, administered close to the temperature minimum, may be of sufficient intensity and length to induce a phase advance. As this group did not compare other wavelengths using the same protocol, they could not assess the differential sensitivity of the circadian system to wavelength.

Our results are consistent with the two studies which showed increasing melatonin suppression with decreasing wavelength [11, 12]. The results are also consistent with the recent report of phase advance produced by short wavelength light [22]. The present study has confirmed that for equal irradiances the shorter wavelengths of light, in particular the blue/green and blue LEDs were the most effective in phase advancing the circadian rhythm. This result, together with our earlier phase delay study [19] demonstrates that the circadian system can be re-timed more effectively with shorter wavelengths of light. It also shows that the longer wavelengths have little, if any, re-timing effect.

One limitation of this conclusion is with respect to the intensities used in both this study and our earlier study [19]. We cannot say whether this same pattern of differential phase delays or advances with the five wavelengths tested will hold for less or greater irradiance than 130 $\mu W/cm^2$. Only a full parametric evaluation of wavelength and intensity on phase change similar to the full action spectra analysis for melatonin suppression [11, 12] would be able to confirm the generally greater sensitivity of circadian phase change to shorter wavelength light. Our choice of irradiance level was an attempt to use an intensity high enough to produce clinically meaningful phase change but not so high as to be poorly tolerated.

Another qualification of our results needs consideration. As we were most interested in phase response under normal physiologic conditions as in a typical clinical situation, we did not predilate the pupils of participants before differential wavelength light exposure. Hence the wavelength effect may be confounded with differential pupillary constriction. For example, if there were greater pupillary constriction to long wavelengths, the effectively decreased retinal irradiance may result in a reduced phase response. However, it has generally been accepted that pupillary responsiveness follows scotopic sensitivity [25] which would result in greater pupillary constriction of the shorter wavelengths in this study. More recent evidence would also suggest less pupillary constriction for equal luminance of longer wavelength light in the yellow to amber region than other wavelengths [26]. Therefore, if pupillary constriction is greater for the shorter wavelengths in the present study, our results would suggest that direct retinal stimulation would show even greater circadian responsiveness to shorter wavelength light.

For a final concluding comment, the light source using LEDs can effectively induce circadian re-timing. Its portability may have advantages over traditional light boxes for treating a range of circadian rhythm disorders particularly those in which a stationary light source requiring mains power would be inconvenient or impossible. However, if the LEDs are to be considered as a therapeutic device in humans, their safety needs to be evaluated as it also needs to be for all bright light devices, most of which emit light over the whole visible spectrum [27].

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