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Phase advancing human circadian rhythms with short wavelength light

Victoria L. Warman*, Derk-Jan Dijk, Guy R. Warman, Josephine Arendt, Debra J. Skene

Centre for Chronobiology, School of Biomedical and Life Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK

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Abstract

The photoreceptor(s) responsible for photoresetting of the human circadian system have not been identified. The aim of the present study was to assess the ability of short wavelength light to alter the timing of circadian rhythms. Eleven male subjects were studied in 15 4-day trials with a single 4 h light pulse administered on day 3, immediately after habitual wake time. The magnitude of the phase shifts in the melatonin acrophase and offset were similar after white ($4300 \mu\text{W}/\text{cm}^2$) and short wavelength ($28 \mu\text{W}/\text{cm}^2$) light exposure even though the white light pulse contained 185-fold more photons than the short wavelength light. This finding suggests short wavelength sensitivity of the photoreceptors mediating synchronization of human circadian rhythms.

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Circadian rhythms are generated by an internal clock and exist to allow anticipation of and appropriate synchronization to rhythmic environmental events that result from the earth's rotation. The human endogenous clock, situated in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus [8], has an intrinsic period that deviates slightly from 24 h and therefore it must be synchronized (entrained) with the 24 h day on a daily basis. The light–dark cycle is the most important environmental stimulus involved in these daily adjustments (phase shifts) of the human circadian system. The size of the phase shift observed in response to a light stimulus depends on the stimulus strength and the circadian time (CT) of administration. Light pulses cause phase delays when administered in the early subjective night and phase advances when administered in the late subjective night/early subjective morning (reviewed in Ref. [5]). However, the retinal photoreceptor(s) responsible for transmitting this photic information from the eyes to the clock have not been identified.

The mammalian eye has parallel outputs that encode either visual images or irradiance information. Photic information for entrainment is conveyed from the retinal ganglion cells (RGCs) to the SCN primarily via the retinohypothalamic tract (RHT) [6]. Studies showing that

transgenic rodless coneless mice exhibit apparently normal circadian responses to light [3] strongly suggest that there is an unidentified circadian photoreceptor(s) present in the inner retina. Three candidate proteins have been located to the inner retina (melanopsin [7] and cryptochrome 1 and 2 [11]) and melanopsin has been shown to contribute to the light-induced phase shifting response in mice [9].

Human studies assessing acute non-visual responses to light suggest that such responses are mediated by an opsin based photopigment [1,10]. Recent investigations into the spectral sensitivity of human circadian photoreception have utilized light-induced melatonin suppression as an approximation for the action of light on the circadian clock. Two action spectra have been constructed, both of which fit an opsin template with a λ_{max} around 460 nm (λ_{max} 464 nm [1]; λ_{max} 459 nm [10]). However, whether a similar short wavelength sensitivity exists for light-induced phase shifting of the human circadian clock remains to be established. To date there has only been a single study investigating the effects of narrow band light on phase shifting [12]. Light of λ_{max} 497 nm was identified as the most effective wavelength for light-induced phase delays. However, light pulses of equal irradiance rather than equal photon density were compared. Furthermore, wavelengths shorter than 470 nm were not studied. Since the action spectra for light-induced melatonin suppression show maximal sensitivity around 460 nm [1,10] these short

* Corresponding author. Tel.: +44-1483-689712; fax: +44-1483-689712.

E-mail address: v.warman@surrey.ac.uk (V.L. Warman).

wavelengths need to be investigated before any firm conclusions can be made.

The present study was designed to test the null hypothesis that the photopic visual system provides the primary input for the phase shifting effects of light on the human circadian system. This implies that the ‘circadian’ strength of the light stimulus can be quantified in photopic lux. If this were true then it would be expected that a low intensity short wavelength light pulse (approximately 8 lux) would be significantly less effective than a high intensity white light pulse (12000 lux) and have as little effect as a dim white light pulse of the same intensity (approximately 8 lux). If, however, the phase shifting effects of light exhibit short wavelength sensitivity, similar to that observed for the light-induced melatonin suppression response [1,10], then it would be expected that a low lux short wavelength light pulse would be as effective as a high lux white light pulse and significantly more effective than a dim white light pulse.

Eleven healthy male subjects, aged between 18 and 40 years (28 ± 5 years, mean \pm SD), participated in 15 phase shifting sessions. Ethical approval for the study was granted by the University of Surrey Ethics Advisory Committee. Subjects were not taking any medication or drugs known to affect melatonin production (e.g. β blockers, benzodiazepines) and were screened for drugs of abuse. In order to reduce the inter-individual variation in circadian phase subjects only participated if they had a regular sleep–wake cycle (onset between 22:00 and 24:00 h and wake between 07:00 and 08:00 h). Subjects had no complaints of sleep disorders (Pittsburgh sleep quality index <5) and were neither morning nor evening types according to the Horne–Östberg questionnaire. Subjects had no colour vision deficiencies according to the Ishihara colour blindness plate test. For 2 weeks prior to the study session subjects were required to keep a regular sleep–wake schedule (23:00–07:00 h) which they could deviate from by up to 30 min in either direction. Compliance was confirmed by sleep diaries and actigraphic recordings (AWL, Cambridge Neurotechnology Ltd., UK).

Each study session lasted 4 days (18:00 h day 1 to 10:00 h day 4) during which subjects were confined to the Clinical Investigation Unit (CIU): baseline 18:00 h day 1 to 12:00 h day 2; light exposure day 3; post-stimulus assessment 16:00 h day 3 to 10:00 h day 4. Throughout the study subjects wore a rectal temperature sensor (Squirrel temperature loggers, Grant Instruments, Cambridge, UK) and the environmental lighting levels in the CIU were maintained at <8 lux. Each day (18:00 h until 12:00 h the following day) the subjects’ posture was controlled to minimize any postural effects on melatonin and core body temperature (18:00–23:00 h and 07:00–12:00 h semi-recumbent; 23:00–07:00 h sleep period, supine and wearing eye masks). During this time blood samples were taken, via an indwelling cannula in the forearm, every 30–60 min. The timing and content of the three daily meals were standardized.

At the end of the second night (i.e. day 3), immediately after habitual wake time, the subjects were exposed to a randomly assigned 4 h light pulse from 07:15 to 11:15 h, timed to induce phase advances. During light exposure subjects were instructed to alternate their gaze every 6 min from looking directly at the light source (90°) to lowering their heads (20° angle to the light). The light source was a light box (Outside-In Ltd., Cambridge, UK) fitted with six fluorescent bulbs (Sylvania CF - LE55W/835, 27916 energy saver Lynx). For the low intensity short wavelength light pulse (8 lux, $28 \mu\text{W}/\text{cm}^2$, 6.21×10^{13} photons/ cm^2/s) subjects ($n = 8$) wore specially designed glasses (Premiere Optical Services, Clacton-on-Sea, UK) with interference filters (Coherent Ealing Europe Ltd., Watford, UK) slotted into the lens space. The short wavelength light pulse had a transmission spectrum with two distinct peaks (λ_{max}) of 436 nm (half-maximal bandwidth $(\Delta\lambda)_{0.5}$ of 2 nm) and 456 nm ($(\Delta\lambda)_{0.5}$ of 5 nm). For the bright white light pulse (12000 lux, $4300 \mu\text{W}/\text{cm}^2$, 1.15×10^{16} photons/ cm^2/s) subjects ($n = 4$) wore the glasses without filters. In the dim white light condition (<8 lux, $<1.6 \mu\text{W}/\text{cm}^2$) subjects ($n = 3$) were exposed to CIU lighting conditions for the 4 h period. Four subjects completed two conditions ($n = 2$ bright white (BW) and short wavelength (SW), $n = 1$ SW and dim white (DW), $n = 1$ BW and DW).

The plasma melatonin and core body temperature rhythms were used to assess circadian phase. Phase shifts were computed by comparing the timing of phase markers on night 3 to night 1. The raw temperature data were converted into hourly moving averages to calculate the temperature nadir (T_{min}) for each night. Four different methods of analysis (cosinor, demasked cosinor, mid-range crossing method and ten lowest values) were used and were well correlated ($R^2 = 0.814$). T_{min} calculated by averaging the times that the ten lowest temperature values occurred each night was selected as the phase marker as it most accurately represented the raw data. The temperature profiles of one of the subjects following a short wavelength light pulse was discarded due to insufficient data and not used in further analysis.

Plasma melatonin levels were measured by RIA (Stockgrand Ltd., University of Surrey, Guildford, UK). The melatonin profiles were analyzed blind by the mid-range crossing method to derive three phase markers of the clock: melatonin onset (Melon50%, time of melatonin onset, 50% maximum levels), melatonin offset (Meloff50%, time of melatonin offset, 50% maximum levels) and melatonin acrophase (time of melatonin peak, midpoint between Melon50% and Meloff50%). The amplitude of the melatonin rhythm was calculated using cosinor analysis. Unpaired Student’s *t*-tests were used to test the hypotheses that there would be no difference between the short wavelength and the dim light pulses but that the bright white light pulse would be significantly better than both the low intensity light conditions. Paired Student’s *t*-tests were used for within subject comparisons.

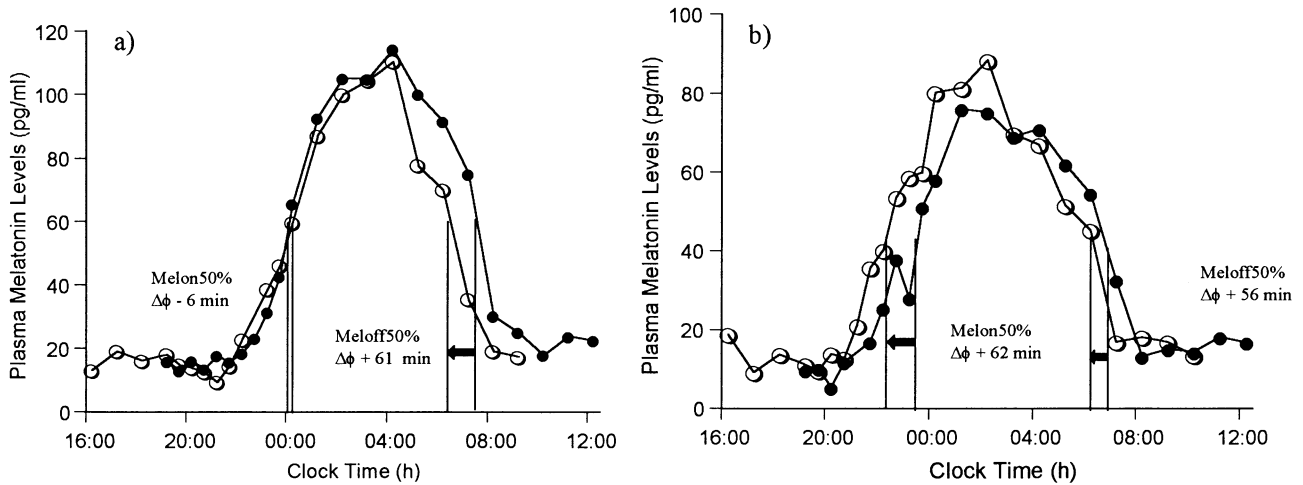


Fig. 1. The effect of 4 h of (a) white light (subject 2) and (b) short wavelength light (subject 4) centred at CT 4.2 on the human melatonin profile (●, night 1, baseline; ○, night 3, after the light pulse). $\Delta\phi$ is the change in phase, + denotes a phase advance, and - denotes a phase delay.

The scheduled sleep–wake cycle during the pre-laboratory segment of the study entrained subjects to the

same circadian phase (night 1 Melon50% 23:38 ± 0:16 h, acrophase 03:14 ± 0:09 h, mean ± SEM, $n = 15$). If the

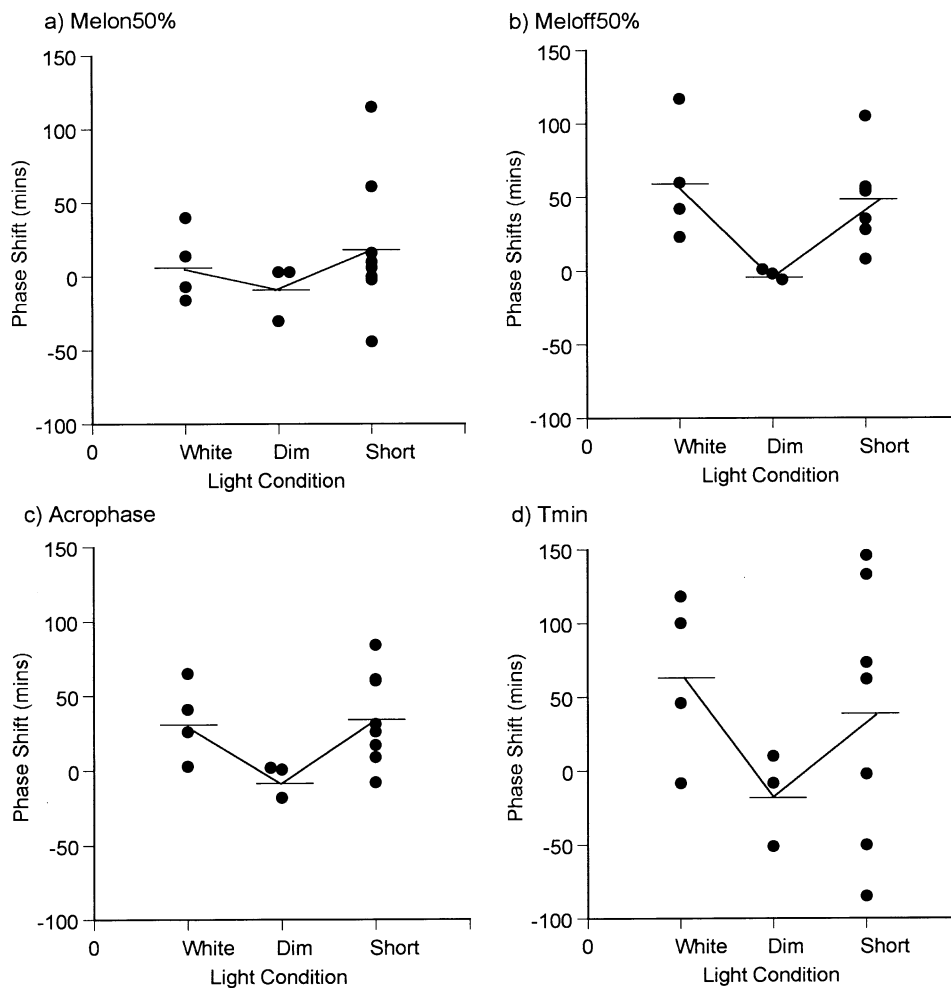


Fig. 2. Phase shifts in circadian phase markers following a 4 h light pulse centred at CT 4.2: (a) melatonin onset (Melon50%); (b) melatonin offset (Meloff50%); (c) melatonin acrophase; and (d) temperature nadir (T_{min}) (●, individual data; —, mean; Short, short wavelength). Positive phase changes indicate phase advances and negative phase changes indicate phase delays.

melatonin acrophase on night 1 is used as a marker of phase and is assumed to occur at CT 22, the midpoint of light administration was $CT 4.2 \pm 0.2$.

Fig. 1 shows an example of the effects of the white and short wavelength light pulse on an individual's melatonin profile. A summary of the phase shifts observed in the melatonin and temperature phase markers is shown in Fig. 2. The short wavelength light produced a phase advance of 51 ± 10 min (mean \pm SEM, $n = 8$) in the Meloff50% and a phase advance of 36 ± 11 min in the melatonin acrophase. Compared to the dim light condition, the short wavelength light was significantly more effective at phase advancing the Meloff50% ($P < 0.02$) and melatonin acrophase ($P = 0.06$). The bright white light pulse also produced phase advances in the Meloff50% (62 ± 20 min, $n = 4$) and in the melatonin acrophase (35 ± 13 min, $n = 4$). This phase shift in Meloff50% was significantly different ($P < 0.05$) from the dim light condition ($n = 3$). The advances in the acrophase and Meloff50% with the bright white light were not significantly different from those observed with the short wavelength light. Comparing the phase shift in Meloff50% with Melon50% within individuals, the size of the phase advance was larger in the Meloff50% than in the Melon50% after both the white ($P < 0.05$) and short wavelength ($P < 0.10$) light. Following short wavelength light exposure the amplitude of the melatonin rhythm was significantly reduced by 24% ($\pm 7\%$) on night 3 compared with night 1 ($P < 0.05$).

Changes in T_{\min} were also observed. Whereas after dim light exposure the timing of T_{\min} was delayed by -15 ± 18 min ($n = 3$), the white and short wavelength light pulses produced phase advances in T_{\min} timing of 65 ± 28 min ($n = 4$) and 41 ± 33 min ($n = 7$), respectively. These differences in T_{\min} were not statistically significant although the shifts were in the same direction as the melatonin data.

Our observation that the Melon50% and Meloff50% do not shift in parallel in response to a phase advancing light pulse has been previously observed following white light exposure [2] with a 6 h morning light pulse advancing melatonin offset of 118 ± 13 min compared to 100 ± 20 min in the melatonin onset. The non-parallel, light-induced phase shifting of the melatonin onset and offset has previously been attributed to the presence of two oscillators, morning (M) and evening (E), which through coupling form the circadian pacemaker [4]. It has been proposed that the melatonin offset, a phase marker for the M oscillator, is more sensitive to phase advancing light pulses whereas the melatonin onset, a phase marker for the E oscillator, is more responsive to phase delaying light pulses. The dual oscillator model fits with our current data in that light administered to phase advance produced a larger phase advance in the melatonin offset (M oscillator).

In conclusion, the current data demonstrate that a very low intensity short wavelength light pulse (8 lux) is able to phase advance the human circadian system to a similar magnitude

as a bright white light pulse (12000 lux) containing 185-fold more photons. This finding suggests that the human circadian system is particularly sensitive to the phase advancing effects of short wavelength light and that the visual photopic system is not primarily involved. Our finding supports the recent human studies investigating the spectral sensitivity of light-induced melatonin suppression [1,10].

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