

Short-Wavelength Sensitivity of the Human Circadian System to Phase-Advancing Light

Dear Editor,

The light-dark cycle is the most important environmental stimulus for entraining the human circadian system (reviewed in Czeisler and Wright, 1999; Mistlberger and Skene, 2004). In mice, all nonvisual light responses are maximally sensitive to wavelengths around 480 nm (Hattar et al., 2003), and in humans, a range of nonvisual light responses has been shown to short wavelengths (Brainard et al., 2001; Thapan et al., 2001; Wright and Lack, 2001; Lockley et al., 2003; Warman et al., 2003; Cajochen et al., 2004; Wright et al., 2004). However, the peak sensitivity of the human phase-shifting response has yet to be determined; in particular, the phase-shifting ability of wavelengths less than 460 nm needs to be assessed. The aim of the present study was to compare the ability of 3 short-wavelength monochromatic light pulses (420, 440, and 470 nm light) to phase advance the human circadian system in comparison to 600 nm light. A representation of the protocol used is shown in Figure 1.

The overall mean times of melatonin onset (Melon50%) and melatonin acrophase on night 1 (N1) were 23.3 ± 0.4 h ($n = 12$) and 3.4 ± 0.4 h ($n = 12$), respectively. The CT of light administration was 3.9 ± 0.4 h ($n = 12$). Figure 2 shows the mean phase shifts in all the phase markers under the 3 high-photon density light conditions. There was a significant difference in the phase advances observed in the SynOff ($F = 4.24$, $df = 18$, $p = 0.03$); phase advances with 470-nm light exposure (74.4 ± 14.8 min) were larger than with 600 nm (-1.3 ± 17.7 min; $p < 0.05$). The 440-nm light exposure gave an insignificant SynOff advance (-2.4 ± 25.8 min; $p = 0.07$), but the error was too large to distinguish its lack of effect from the clear advance with 470 nm. The remaining markers also showed a greater advance at 470 nm compared to those seen using 440

nm or 600 nm, but the differences were not statistically significant. In general, phase shifts were small and variable, and there were essentially no phase shifts at 440 or 600 nm (Fig. 2). With low-photon density, there were, at best, paltry phase shifts, inconsistent in direction, and no significant differences with wavelength (data not shown).

Our results indicate that the 470-nm light is more effective than the 600-nm and, perhaps, the 440-nm light in phase advancing the offset of the human melatonin rhythm. This is the 1st time that wavelengths shorter than 460 nm have been assessed for their phase-shifting ability. The results of the current study, combined with previous work (Wright et al., 2004), suggest that the phase-advancing response exhibits a similar spectral sensitivity to that observed for light-induced melatonin suppression (Brainard et al., 2001; Thapan et al., 2001), with a maximal sensitivity between 460 and 480 nm, and may involve the same proposed novel opsin-based photopigment (Hankins and Lucas, 2002). The clarity of the results from this study was likely limited by the small sample size and by the unequal impact of each subject on the data set. Furthermore, before final conclusions can be drawn, it will be necessary to complete a full-action spectrum using the same subjects for each wavelength tested.

The melatonin onset and offset responded differentially to the morning light exposure, as was observed in our previous study (Warman et al., 2003), and this may reflect the putative morning and evening oscillators (Pittendrigh and Daan, 1976). However, the phase advances observed in the Meloff50% are smaller (approximately half) than those observed in our previous study (Warman et al., 2003). This is likely due to the intermittent rather than continuous exposure and the use of monochromatic light rather than a broader range of wavelengths (432-462 nm; Warman et al.,

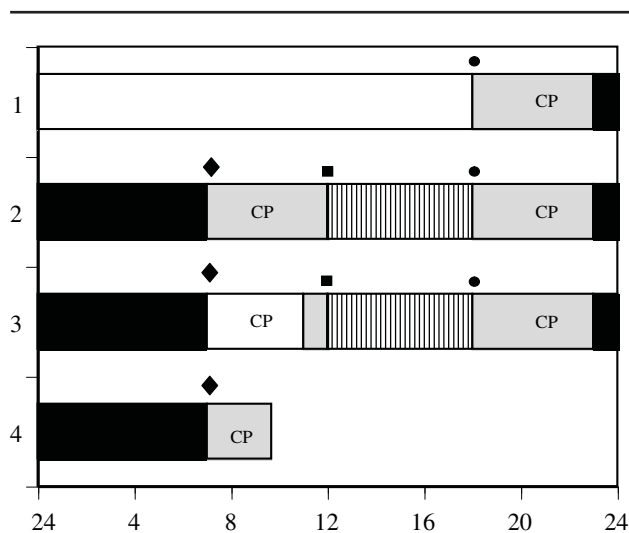


Figure 1. Diagram representation of the 4-day protocol: night 1 (N1) was a baseline assessment, night 2 (N2) included the light stimulus, and night 3 (N3) was for poststimulus assessment. Black bars represent sleep period (2300-0700 h) with subjects supine and wearing eye masks in 0 lx, gray bars represent wake time in < 8 lx, bars with vertical stripes represent free time when subjects chose their activity in < 8 lx, the white bar represents the 4-h light stimulus (0715-1115 h), and CP indicates periods of controlled, semi-recumbent posture when blood sampling occurred. Breakfast at 0700 h (diamonds), lunch at 1200 h (squares), and supper at 1800 h (circles).

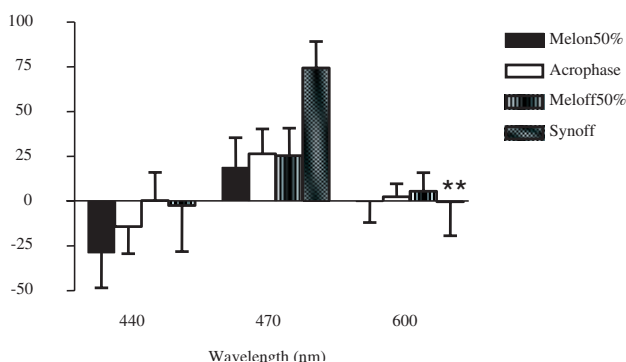


Figure 2. Phase shifts in minutes (mean \pm standard error of the mean [SEM]) of melatonin phase markers for the 3 high-photon density light pulses (440, 470, and 600 nm). Phase shifts were calculated by comparing extrapolated N3 values to measured N3 values. $**p < 0.05$ compared to 470-nm light.

2003). It is possible that the magnitude of the phase advances observed may underestimate the final absolute change in phase, as it was determined in the cycle immediately following stimulus application, while the clock may have been undergoing transiency before reaching a steady state (Pittendrigh, 1965). Despite this, the SynOff showed spectral differences

in the magnitude of phase advances the day after the light exposure. Thus, the SynOff may more accurately represent the phase of the clock than the Meloff50% phase marker, which occurs at a lower point on the melatonin profile and may be influenced by, for example, metabolism rates.

APPENDIX MATERIALS AND METHODS

Subjects. Twelve male, drug-free subjects, ages 27 ± 4 years (mean \pm standard deviation [SD]), were recruited according to previously defined criteria (Warman et al., 2003). The protocol was approved by the University of Surrey Ethics Committee. The 4-day phase advance protocol (Fig. 1) was modified slightly from that previously described (Warman et al., 2003). A 4-h monochromatic, intermittent light pulse was administered on day 3, immediately after habitual wake time. The light was centered at CT 4 and therefore scheduled to produce phase advances, according to published phase-response curves (e.g., Khalsa et al., 2003). Complete overnight profiles of melatonin blood levels suitable for temporal analysis and comparison were obtained in 30 sessions.

Lighting equipment. Four monochromatic wavelengths were tested at 2 photon densities: 420 nm and 440 nm at 2.3×10^{13} photons/cm²/sec and 440 nm, 470 nm, and 600 nm at 6.2×10^{13} photons/cm²/sec. The range of light intensities used was 11 to 28 μ W/cm² or 0.7 to 17.5 lx. Subjects placed their heads in a specially designed visor (20 \times 10 \times 10 cm) that delivered monochromatic light from the center of its rear surface. The light source was a PL900 light box fitted with a 150-W quartz halogen bulb (Dolan Jenner Industries, Lawrence, MA) delivering light via a fiber-optic cable (Edmund Optics, York, UK). The monochromatic filters (12.7 mm diameter, Coherent Ealing Europe Ltd., Watford, UK) had half-maximal bandwidths ($\Delta_{0.5}$) of < 5 nm. Uniform distribution was ensured by a single layer of diffuser paper, and irradiance was adjusted using Kodak Wratten neutral density filters (Richard Frankfurt, Croydon, Surrey, UK). The spectral distribution was confirmed using a Spectrascan 650 portable spectrometer (Photoresearch, Chatsworth, CA). The visor was positioned so subjects could move it toward their face for the 10-min light exposure and push it away (90° rotation) for the 5-min dim-light period (< 8 lx). This cycle was repeated 16 times during the 4-h trial. The number of subjects studied in each light condition was as follows: 2.3×10^{13} photons/cm²/sec at 420 nm ($n = 4$) and 440 nm ($n = 5$) and 6.2×10^{13} photons/cm²/sec at 440 nm ($n = 6$), 470 nm ($n = 6$), and 600 nm ($n = 9$). Four subjects received 1 light condition, 4 subjects received 2 light conditions, 1 subject received 3 light conditions, and 3 subjects received 5 light conditions.

Calculations. SynOff, which estimates the point at which pineal melatonin synthesis is switched off (Lewy et al., 1999), was estimated by an Excel-based algorithm (Terman, 2000) tailored for the sampling interval of this study.

Melatonin concentrations (pg/mL) were \log_{10} -transformed, and 2 linear regressions ("pre" and "post") were iteratively fitted to the data using candidate SynOff points throughout the data range beginning at 0200 h and ending at 1100 h. Linear regressions were compared for 4-h windows on either side of the test point, and the maximum slope difference in the set was chosen to indicate the 1-h interval encompassing the SynOff (i.e., the region in which melatonin concentration begins its exponential descent). The 2 regression lines were then extrapolated to their intersection and the SynOff estimated to the nearest 3 min. In addition, 3 melatonin phase markers were calculated by the midrange crossing method (Melon50%, Meloff50%, and acrophase; Warman et al., 2003). Phase shifts were calculated by comparing the timing of the measured night 3 (N3) phase markers with the extrapolated N3 phase markers based on the individual drift (7 ± 6 min, mean \pm standard error of the mean [SEM]) from night 1 (N1) and night 2 (N2) in dim-light conditions.

The high-photon density light data (6.2×10^{13} photons/cm²/sec) were analyzed by 1-way analysis of variance followed by Tukey-Kramer multiple post hoc comparisons. Unpaired Student *t* tests were used to compare the 2 low-photon density light pulses (2.3×10^{13} photons/cm²/sec). An α of 0.05 was set as the criterion for significant difference. Data are expressed as mean \pm SEM.

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