

Entrainment of the human circadian pacemaker to longer-than-24h days

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SUPPORTING INFORMATION

SUPPORTING RESULTS

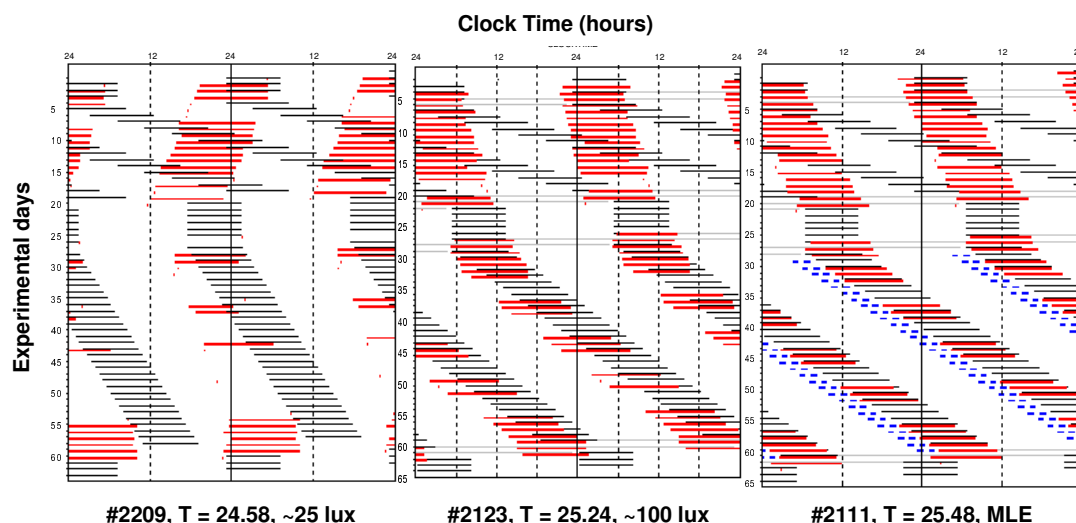


Figure 6: Dynamics of the melatonin rhythm in three individual subjects during their 65 day study. Plasma melatonin levels that were higher than the MEL_{on} are illustrated by the red horizontal bar. The forced-desynchrony segment reveals the period (τ) of the subjects' circadian pacemakers. The entrainment segment was subject-adapted ($T = \tau + 1h$). These data demonstrate failure to entrain the circadian pacemaker to $T = \tau + 1h$ when exposed to 25 lux (*left*), entrainment with a large phase angle (wider ψ_{MELon} and ψ_{MELoff} compared to baseline) when exposed to 100 lux (middle), and entrainment with a small phase angle when exposed to MLE (*right*). Note that subject 2111's melatonin secretion was consistently restricted to the dark phase of the T-cycle.

SUPPORTING MATERIAL AND METHODS

Subjects:

Subjects had no medical, psychiatric, or sleep disorders and were drug free. Subjects' screening included physical examination, psychological screening questionnaires and a structured interview by a clinical psychologist, electrocardiogram, blood and urine biochemical screening tests, and a comprehensive toxicological analysis for drug use conducted at admission to the laboratory.

Lights characteristics:

The experimental suites were equipped with ceiling-mounted cool-white fluorescent lamps [Phillips (Eindhoven, The Netherlands) T8 and T80]. Clear polycarbonate lenses filtered 99.9% of the light in the UV range from the light source. In addition subject wore clear goggles during light exp (model #S379; luminous transmittance 90%, UV absorption >99%, Uvex Safety, Smithfield, RI, USA).

The *correlated color temperature* of the lamps used in our study is given to be 4,100 K. The color temperature of a lamp is an indicator of the perceived color of a lamp, however, it has no informative value on its spectral distribution curve. Therefore, we are giving in Fig. 8 the spectrum of the lamps used in our study. For information, "the term *color temperature* is applied to highly selective radiators, such as electric discharge lamps, when the light of this radiator has the same (or nearly the same) chromaticity coordinates as a blackbody radiator at a certain temperature; this temperature is then called the *color temperature* of the selective radiator" (1). For an incandescent lamp - considered a blackbody radiator - its color temperature (in Kelvin) is close to the temperature of its tungsten filament. For a fluorescent lamp, however, the term *correlated color temperature* (CCT) is used

because its chromaticity is not exactly equal to any of the chromaticities of a blackbody radiator. It is defined as the temperature of the blackbody radiator whose perceived color most closely resembles that of the lamp at the same brightness. As examples of color temperatures: a candle: 1850 K, sunrise/sunset: 3200 K, sunny daylight: 5000-6500 K, blue sky: 9000-12000 K.

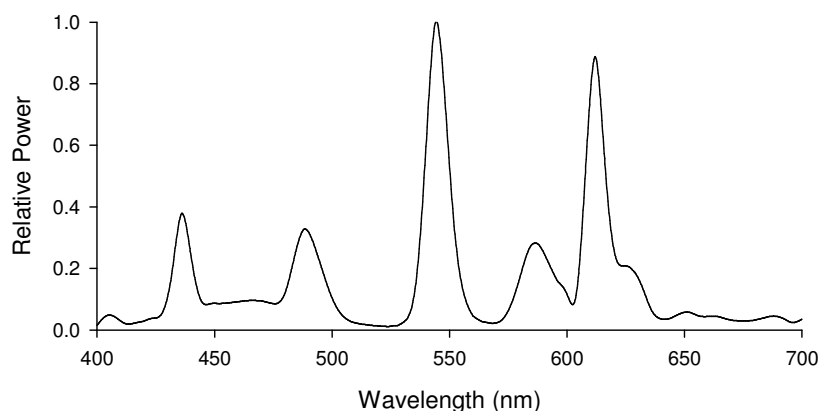


Figure 7: Relative spectral power distribution curve of the lights used in the study. This average spectrum was derived from spectra measured in 41 different locations across the 4 experimental suites studies were conducted in. Measures were done with a PR-650 SpectraScan Colorimeter with a CR-650 cosine receptor (Photo Research Inc., Chatsworth, CA) in a lighting condition calibrated to be of ~ 100 lux ($\sim 30 \mu\text{W}/\text{cm}^2$). The average intensity (photopic) measured was 106 lux and the average irradiance (between 400-700 nm) was $29.9 \mu\text{W}/\text{cm}^2$.

Light conditions:

Light intensities (photopic lux) and irradiances ($\mu\text{W}/\text{cm}^2$) were measured using an IL1400 photometer with a SEL-033/Y/W or a SEL-033/F/W detector respectively (International Light, Newburyport, MA) placed horizontally at a height of ~ 1.37 m to give the intensity received in the average angle of gaze [AG] during wakefulness. An additional measure was taken vertically with the light sensor in the direction of the ceiling fixtures to measure the maximum intensity a subject could be exposed at a height of ~ 1.83 m.

A computer system automatically turned the lighting to the required pre-determined intensity at the scheduled times. Light intensities were: ~ 90 lux [AG] ($\sim 27 \mu\text{W}/\text{cm}^2$, < 190 lux maximum) during the first 2.5 baseline days (Days 1-3); ~ 1.5 lux [AG] ($\sim 0.5 \mu\text{W}/\text{cm}^2$, < 8 lux maximum) during the last 6 hours of day 3, during constant routines (CR1-4) and the forced-desynchrony segment; ~ 450 lux [AG] ($\sim 120 \mu\text{W}/\text{cm}^2$, $< 1,500$ lux maximum) during the re-entrainment segment between CR2 and CR3; and depending on the lighting condition, subjects were exposed to either ~ 25 lux [AG] ($\sim 8 \mu\text{W}/\text{cm}^2$, < 80 lux maximum), ~ 100 lux [AG] ($\sim 30 \mu\text{W}/\text{cm}^2$, < 250 lux maximum) or a modulated light exposure (MLE) protocol consisting of dim light of 25 lux ($\sim 8 \mu\text{W}/\text{cm}^2$) for the first 10h of the waking day, room light of 100 lux ($\sim 30 \mu\text{W}/\text{cm}^2$) for the remainder the waking day plus exposure to two bright light pulses of $\sim 9,500$ lux [AG] ($\sim 2,900 \mu\text{W}/\text{cm}^2$) (see Figure 8).

Consistency of light exposure between subjects was ensured by having subjects seated during the light exposure sessions and instructing subjects to fix their gaze on a target mounted on the wall of the suite for 5 of every 10 minutes. Free gaze around the room was allowed for the remaining 5 minutes. Light intensity measurements, in the direction of gaze, were taken every five minutes to ensure that subjects were exposed to the appropriate light intensity for the appropriate duration.

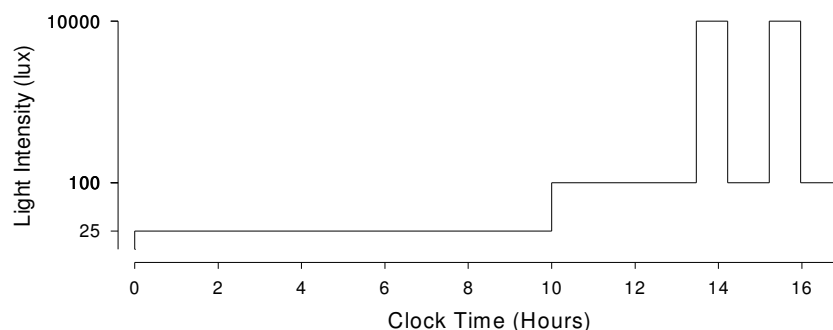


Figure 8: Detail of the modulated light exposure (MLE) condition: during the $\tau+1$ h segment of the study, subjects in this group were exposed to ~25 lux ($8 \mu\text{W}/\text{cm}^2$) during the first 10 h after WT, and subsequently to ~100 lux ($\sim 30 \mu\text{W}/\text{cm}^2$) followed by two 45-min bright light pulses of ~9,500 lux ($\sim 2,900 \mu\text{W}/\text{cm}^2$) separated by 60 min of ~100 lux. Subjects in the two other groups, 25 lux and 100 lux, were exposed during the wake episodes to constant light of ~25 and ~100 lux respectively.

SUPPORTING EXPANDED DISCUSSION

Expected range of entrainment

The entraining stimuli used in our study fall into two categories. The first category is where light is uniform throughout the enforced waketime (either 25 lux or 100 lux) and complete darkness is imposed during enforced bedrest. The second category is where stronger light is used intermittently during those hours for which phase delay is expected from PRC data (2). For the first category, “morning” light is expected to advance circadian phase while “evening” light will contribute to delay shifts. Since a net delay of 1 h is needed ($T = \tau+1$ h), this may be achieved by shifting the pacemaker cycle relative to the light/dark pattern so as to increase the amount of time the delaying phases are illuminated and simultaneously reducing the time for which the advancing phases are illuminated. Another investigator, using ~4000 lux as the background illumination during daytime, has reported entrainment to T cycles between 19-31h (3, 4). Considering the nature of the protocol used and the circadian markers employed in those studies, such ranges may be artifactually large.

A special example of the second category, for which entrainment to $T = \tau+1$ h might be achieved, is where negligible photic stimulus is applied during the phase-advancing hours while a strong, extended pulse of light is applied during phase-delaying hours. From the recently published PRC for a 6.7 h pulse of 10,000 lux (2) where the maximum reported delay is ~3.5 h we might anticipate that subjects with an average of τ of 24.2 h would entrain up to $T = 24.2 + 3.5 = 27.7$ h with a daily 6.7 h pulse of such bright light. Conversely, since those PRC data showed a maximum phase advance of ~2 h, entrainment to T as low as $24.2 - 2 = 22.2$ h might be expected.

Should we oppose continuous and discrete models of entrainment?

Two conceptual models have been proposed to explain entrainment of the circadian timing system. The continuous model of entrainment (5) proposes that angular velocity of the circadian period is modulated continuously (accelerated or decelerated) throughout the circadian day by changes in light intensity. Such effects on period have been suggested to contribute to stabilization of the phase angle difference (6-8). The discrete model (6) proposes that the circadian system is in equilibrium with the T cycle when light elicits the daily resetting necessary to correct for the difference between the endogenous period (t) and the T cycle, accordingly to a phase response curve (PRC). The two models have been largely opposed to each other in the literature. Interestingly, however, most of the results explained by the continuous model, can be explained equally well by the discrete model. An interesting approach was recently proposed by Beersma et al., that period change in response to light (τRC) would improve accuracy of the circadian system (9). Our data could be solely explained by the discrete model, they do not exclude, however, the possibility that a change in period contributed to entrainment.

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