

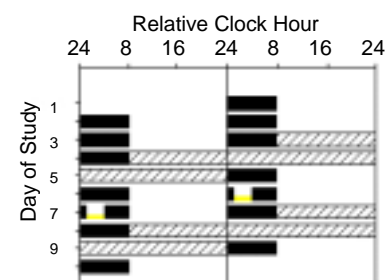
## Supplemental material for K. P. Wright Jr., C. A. Czeisler: Absence of Circadian Phase Resetting in Response to Bright Light Behind the Knees

In (S1), during episodes of exposure to light behind the knees, subjects were concurrently exposed to ocular light in the angle of gaze that reportedly did not exceed 20 lux. This was presumably based on the assumption that such light levels would not elicit significant circadian phase shifts in humans. However, analysis of archival data from 15 subjects of Zeitzer et al. (S2) using the same dim light melatonin onset (DLMO<sub>25%</sub>) phase assessment technique used in the current study revealed that ocular light exposure of ~12 lux significantly phase advanced the melatonin rhythm ( $0.36 \pm 0.43$  h, Mean  $\pm$  SEM) compared to total darkness ( $-1.34 \pm 0.31$  h) ( $p = 0.006026$ ).

A number of groups have conducted experiments to further evaluate the neuroendocrine and therapeutic potential of dermal light exposure following (S1) and have reported that—unlike ocular light exposure—dermal light exposure does not elicit melatonin suppression (S3-S6). Furthermore, exposure of the chest and abdomen to broad-spectrum bright white light did not elicit a circadian phase shift (S7). Moreover, on a different protocol, two episodes of bright light exposure to the back of the knees did not facilitate circadian adaptation in a simulated shift work-study (S8). In addition, neither clinical improvement of mood nor a circadian phase shift was observed in seasonal affective disorder patients treated with bright light to the back of the knees for five days in a outpatient study in which daytime ocular light exposure was not controlled (S9), although the sleep-wake rhythm of a blind, enucleated patient was reportedly re-entrained via white light behind the knee, according to an uncontrolled case report (S10). It should be noted that extraocular circadian photoreception, which has been reported in birds and reptiles (S11-S12), is thought to be lost in mammals (S12), although the pineal of newborn rats (S12) and congenitally anophthalmic mutant rats (S13) has been reported to be photosensitive.

**Materials and Methods.** Ten women and twelve men ( $28.63 \pm 9.4$  (SD) years) gave written informed consent to participate in research procedures that were approved by the Brigham and Women's Hospital/Partners Health Care Human Research Committee. Participants passed a rigorous health screening consisting of medical history, physical and psychological exams, blood and urine chemistries and electrocardiogram. None reported shift work for the past three years or transmeridian travel in the previous three months. Participants maintained a routine of 8-h of scheduled sleep at a consistent clock time ( $\pm 30$  min) for three weeks at home prior to laboratory procedures, verified by sleep logs, phone calls of bedtime and waketime to a time stamped voice recorder and for at least one week via wrist actigraphy (Minimitter, Bend, OR). Toxicology screens verified that participants were drug free upon admission to the laboratory. Ceiling-mounted fluorescent lamps (T8 and T80 lamps, Philips Lighting Company, Eindhoven, NE) with a 4100 K color temperature produced a spectrum of white light. Lighting conditions were ~3.0 lux in the angle of gaze for baseline day 1 and ~150 lux on days 2-3. The baseline days and the maintenance of a strict schedule prior to admission were designed to reduce inter-individual variability in circadian phase and light exposure history prior to the initial circadian phase assessment.

Each subject's wake time following the initial laboratory baseline night was arbitrarily assigned a relative clock hour value of 0800 and all other times were referenced to this value, modulo 24 hours (e.g., breakfast scheduled 1 hr after awakening would be reported as occurring at a relative clock hour of 0900). Sleep was scheduled for 8 h at habitual bedtimes for three initial baseline days (Fig. S1). This was



**Fig. S1.** Double raster plot of phase shifting protocol with scheduled sleep assigned a relative value of 2400 h on baseline day 1. Subsequent days are plotted next to and beneath the other. Dark bars represent scheduled sleep/darkness. Downward diagonals represent constant routines. Small yellow bar represents light exposure to the knee.

followed by a 40-h constant routine (S14) to assess melatonin phase and then by an 8-h recovery sleep. On night 6, subjects were scheduled to light interventions. In order to evaluate the resetting response to light behind the knee, we chose to assess a group of subjects at one circadian phase rather than individual subjects across circadian phases. Therefore, we chose a phase at which substantial phase delays were anticipated based on (S1).

The circadian phase of the intervention, which began one hour after bedtime, was similar in relation to the core body temperature minimum (CBT<sub>min</sub>) (S14) for all three conditions: DK—darkness to the knees and eyes (n=8) was centered  $-3.35 \pm 1.09$  h (mean  $\pm$  SD; range: -5.47 to -1.96 h) prior to CBT<sub>min</sub>; BK—bright light behind the knee (n=7) was centered  $-3.24 \pm 1.35$  h (range: -5.19 to -1.69 h) prior to CBT<sub>min</sub>; and BE—bright light to the eyes (n=7) was centered  $-3.11 \pm 1.18$  h (range: -4.62 to -1.0 h) prior to CBT<sub>min</sub>. Subjects in all three groups were awakened 20 minutes after scheduled sleep time and remained in a supine posture. Technicians wore generation 3 night vision goggles (Night Quest 5001, ITT Night Vision, Roanoke VA) with infrared illuminators to place flexible fiber optic woven pads (Biliblanket® Plus Phototherapy System, Ohmeda, Inc, Madison, WI) behind the knees of subjects in all three groups. This procedure began 20 minutes prior to the beginning of the experimental light exposure session. Night vision equipment was not used during the 3 h light exposure session. Wakefulness of subjects was maintained via verbal interaction and was also continuously monitored via EEG. A second constant routine (days 8-9) was used to assess circadian phase after these interventions. T-tests were used to test for differences in the amount of phase shift between conditions. Biliblanket® Plus fiber optic pads were placed behind the knees, wrapped with ace bandages and covered with blackout material to prevent light leakage. The device which housed the light source for the Biliblanket® Plus Phototherapy System was placed in a ventilated baffling box that prevented light from escaping the housing. For subjects in group DK and BE, the fiber optic cable between the light source and the woven fiber optic pad was blocked by an opaque shield that did not pass light to the skin, although the halogen lamp and fan in the device that housed the light source were still powered on. A photodiode sensor continuously measured light in each housing unit to ensure the lamps were illuminated. Experimental conditions remained double blind until after melatonin data were analyzed except for two subjects, from whom very faint light was observed escaping the blackout shades. A research assistant blind to condition (JTH) analyzed all melatonin phase estimates.

Plasma melatonin was assayed using an <sup>125</sup>I radioimmunoassay technique (Elias USA, Inc., Osceola, WI). The sensitivity of the assay was 2.5 pg/ml. The average inter-assay and intra-assay coefficients of variation were 15.6% and 4.5% respectively. Salivary melatonin data were used for one subject due to missing plasma data. Data from the night of the intervention compared with the night prior was used to evaluate acute effects. In 4 out of 16 of the subjects (2 in DK, 0 in BE and 2 in BK), data from two nights prior to the intervention were used as baseline. Data were z-score transformed and interpolated to provide equal sampling times. The trapezoidal area under the curve (AUC) was calculated for the intervention time (S2). Repeated measures ANOVA with Bonferroni corrections were used to test for AUC differences across nights and between conditions. Missing data for 5 subjects during the intervention reduced the sample size for the acute effects analysis.

Data provided in column P Fig 1A. show the change in phase expected to occur in response to the scheduled dim light-dark/wakefulness-sleep cycle between constant routine circadian phase estimates. Data points represent the cumulative drift in circadian phase due to individual differences in intrinsic circadian period derived from of a group of 10 subjects studied under the same scheduled sleep-wakefulness and lighting conditions (S15) that occurred between constant routines in the current study. These estimates were calculated by multiplying the sum of the individual's intrinsic circadian period minus 24.0 by the number of days in between circadian phase assessments.

During the post-intervention sleep episode, melatonin levels appeared to be lower for DK and BK conditions, though, due to data loss we have insufficient subjects to test for the significance of this effect within each condition separately. However, pooling data from conditions DK (n=3) and BK (n=5), we observed that melatonin levels were significantly lower ( $p = 0.009872$ ) in the 4 hours following the intervention (AUC  $0.81 \pm 0.42$ , mean  $\pm$  SEM) compared to baseline the night prior (AUC  $3.64 \pm 0.56$ ), possibly suggesting an effect of subsequent sleep on melatonin levels.

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