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Randomized Trial of Polychromatic Blue-Enriched Light for Circadian Phase Shifting, Melatonin Suppression, and Alerting Responses

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ABSTRACT

Wavelength comparisons have indicated that circadian phase-shifting and enhancement of subjective and EEG-correlates of alertness have a higher sensitivity to short wavelength visible light. The aim of the current study was to test whether polychromatic light enriched in the blue portion of the spectrum (17000 K) has increased efficacy for melatonin suppression, circadian phase-shifting, and alertness as compared to an equal photon density exposure to a standard white polychromatic light (4000 K). Twenty healthy participants were studied in a time-free environment for 7 days. The protocol included two baseline days followed by a 26-h constant routine (CR1) to assess initial circadian phase. Following CR1, participants were exposed to a full-field fluorescent light (1×10^{14} photons/cm^2/s, 4000 K or 17000 K, n=10/condition) for 6.5h during the biological night. Following an 8 h recovery sleep, a second 30-h CR was performed. Melatonin suppression was assessed from the difference during the light exposure and the corresponding clock time 24 h earlier during CR1. Phase-shifts were calculated from the clock time difference in dim light melatonin onset time (DLMO) between CR1 and CR2. Blue-enriched light caused significantly greater suppression of melatonin than standard light ((mean $\pm$ SD) 70.9$\pm$19.6% and 42.8$\pm$29.1%, respectively, $p<0.05$). There was no significant difference in the magnitude of phase delay shifts. Blue-enriched light significantly improved subjective alertness ($p<0.05$) but no differences were found for objective alertness. These data contribute to the optimization of the short wavelength-enriched spectra and intensities needed for circadian, neuroendocrine and neurobehavioral regulation.

Circadian rhythm

Alertness

Melatonin

1. Introduction

The human circadian pacemaker is exquisitely responsive to ocular light exposure [1]. Photic information is transduced by the retinohypothalamic tract to the suprachiasmatic nucleus (SCN) and then to the pineal gland via a multisynaptic pathway [2]. The SCN-pineal, but not the retinae-SCN pathways need to be intact for the secretion of melatonin, even in the absence of light [3,4]. By way of this neuroanatomy, cycles of light and dark which are detected through the eyes entrain SCN neural activity which, in turn, entrains the rhythmic synthesis of multiple physiological, endocrinological, metabolic and behavioral systems including melatonin secretion from the pineal gland [5].

In virtually all species including humans, high levels of melatonin are secreted during the night and low levels are secreted during the day [6]. Ocular light exposure affects melatonin secretion in two major ways –light exposure during the biological night causes the acute suppression of melatonin [7] and light exposure throughout the 24-hour day shifts its rhythm according to a Phase Response Curve [8]. The strong circadian control of melatonin and its sensitivity to light timing mean that melatonin is often used as a marker of the circadian clock [9].

Light is detected primarily by a specialized subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) [10,11,12,13]. These non-rod, non-cone photoreceptors express

the photopigment melanopsin which has a short wavelength peak sensitivity around 480 nm [14,15].

Using this defined spectral sensitivity of the melanopsin photopigment in ipRGCs, multiple studies have examined the spectral sensitivity of melatonin suppression, circadian phase shifting, and the acute effects of alertness using narrowband (15 nm or greater halfpeak bandwidth) or monochromatic (less than 15 nm halfpeak bandwidth) light sources. Monochromatic blue light has been shown to cause greater circadian phase shifting and acute alerting responses over monochromatic green light [16,17,18,19,20].

Studies have also documented short-wavelength sensitivity for neuroendocrine effects using broad spectrum polychromatic blue-enriched light evoking stronger melatonin suppression than those with relatively less short-wavelength light [21,22]. In both of these studies, equal photopic lux bright light exposures of either 1,000 lux [22] or 2,500 lux [21] at high CCT (6480-6500K) caused greater increases in melatonin suppression and increases core body temperature than low CCT (3000-3150 K) broad spectrum polychromatic light. Sato and colleagues reported that a 2 hour light exposure in the early morning caused significant acceleration in the rise of core body temperature and fall of salivary melatonin secretion from the blue-enriched, high CCT condition [21]. Evening light exposures of 5 hours were employed by Morita and Tokura causing significant suppression of the nocturnal rise urinary melatonin and the fall of core body temperature in the high CCT group [22]. These carefully done initial studies, however, were limited in that they examined only the acute neuroendocrine effects of relatively intense polychromatic light sources. We built on these results to examine both acute and circadian neuroendocrine

responses as well as neurobehavioral differences using a standard light source at more common lower room illuminances

In addition to highly controlled laboratory studies, several real-world applications in schools and offices using both static and dynamic lighting manipulations involving blue-enriched light have demonstrated increased subjective measures of alertness and affect as well as improvements in standard tests of cognitive processing speed, concentration and reading ability in blue-enriched lighting conditions[23,24,25,26,27]. For example, office installation of the fluorescent lamps used in this study on two floors with participants being exposed in a counter-balanced order resulted in statistically significant improvements in self-reported measures of alertness, mood, performance, evening fatigue, irritability, concentration and eye discomfort during the month of 17000 K exposure as compared to 4000 K [23]. Mott et al. (2012) studied third grade students (age 7-8 years) under the Normal (500 lux, 3500K) versus Focus (1000 lux, 6500K) light conditions and found that the higher CCT lighting was reported to improve oral reading fluency when assessed over a full calendar year [25].

Recently, we conducted a within-subjects study that established full-range fluence-response curves to three types of fluorescent lamps that differed in their relative emission of light in the short wavelength end of the visible spectrum between 400 and 500 nm demonstrating that increasing corneal irradiances of light evoked progressively increasing suppression of nocturnal melatonin. Comparison of these fluence-response curves supports the hypothesis that polychromatic fluorescent light is more potent for melatonin regulation when enriched in the short wavelength spectrum [28]. Building on these data, we tested the hypothesis that blue-enriched polychromatic fluorescent light (17000 K) can be more

effective than standard white fluorescent light (4000 K) light for eliciting melatonin suppression, enhancement of neurobehavioral function and phase shifting in healthy human participants. This carefully controlled comparison of a high color temperature polychromatic light source was performed under commonly occurring room illuminances while balancing for photon density.

2. **Materials and Methods**

2.1 Participants

A total of 11 males and 9 females (mean age $\pm$ SD of 22.1 $\pm$ 1.8 years, range -19 to 26 years) were studied in the Light Research Laboratory of Thomas Jefferson University between July 2007 and August 2008. The study was approved by the Institutional Review Board of Thomas Jefferson University and all participants gave written informed consent prior to entering the protocol. All participants underwent psychological, toxicological, physical, and ophthalmological exams including color vision testing. For at least 10 days prior to entry into the seven-day protocol participants maintained a self-selected 8-hour sleep/wake schedule with calls to a time- and date-stamped voicemail at bedtime and wake. Participants also wore an actigraph (BASIC Motionlogger, Ambulatory Monitoring, Inc., Ardsley, NY) to verify bed and wake times during this time. The exclusion criteria included no use of recreational, over-the-counter, or prescription medications inclusive of caffeine and alcohol in the 10 days prior to admission. Toxicological screening for drugs of abuse, caffeine and alcohol as well as pregnancy testing, basic metabolic panel, thyroid function, and a complete blood count was conducted during screening and on the day of entry in the facility. Both groups were gender balanced (5M, 5F in 17000 K group and 4M, 6F in 4000 K group) and the testing of two sleep parameters from actigraphy revealed no

differences (Total Sleep Time and Awakenings) from actigraphy revealed no differences (p=0.23 and p=0.70, respectively).

2.2 Protocol design

Figure 1 provides a diagrammatic representation of the 7-day inpatient protocol. This protocol was adapted from the 9-day protocol used at the Brigham and Women's Hospital [16]. Participants lived in a time-free environment (no windows, timepieces, TV, or internet access with continual supervision by trained staff) and remained in their assigned room for the duration of the study. During the constant-routine, participants remained awake in dim light (< 5 lux) in a semi recumbent position while under direct supervision with isocaloric meals (basal energy expenditure x 1.3) being served every two hours.

2.3 Study lighting conditions

Ambient light in the facility was produced by ceiling-mounted standard cool-white (4100 K CCT) 40 W fluorescent lamps (F40T12/841ALTO, Philips Lighting, B.V., Eindhoven, Netherlands) placed above matte white acrylic 0.080-inch diffuser panels and controlled by a full range dimming system (Model #HLT-2000/3 Dimmer Module with HILUME Electronic dimming ballast OPSCU Series, DP-1 dimmer panel, VA-3-4A Control Panel, Lutron Corp., Coopersburg, PA). The light intensities in the study rooms were < 80 lux maximal (at a height of 122 cm in the vertical plane) on study day 1 and during the first eight hours of study day 2. For the rest of the waking hours of the study, participants were kept in constant dim light with illuminances < 5 lux in the vertical plane. Eight-hour sleep opportunities scheduled at the average of the 10 days prior to admission occurred in

complete darkness. Daily illuminance/irradiance measures were taken using an IL-1400BL radiometer/photometer (International Light Technologies, Inc., Peabody, MA). This meter had either a model SEL033/Y/W detector or to provide photopic illuminance response or SEL033/F/W to provide flat irradiance response and each was annually given a full scanned calibration. All calibrations were traceable to the U.S. National Institute of Standards and Technology.

Experimental polychromatic light exposures occurred on the night of study day 4 when participants underwent a 6.5-hour light exposure (LE) that began 9.25 hours before their habitual wake time. Light exposure was timed to correspond to approximately 6.75 h before core body temperature minimum, a phase where previous studies produced phase delays [16,19]. The 4' x 4' light exposure system held both sets of 54 W fluorescent lamps in each. A diffuser cover on the front of the panel allowed for a uniform field of light exposure for the subject. The panel was attached to an analog dimmer switch to adjust intensity. The lamps, as well as the lighting panel unit, were constructed and donated by Philips Lighting, B.V. (Eindhoven, Netherlands). An additional neutral density filter panel (0.3 ND 50% transmission) that covered the entire light panel surface was used to further adjust the intensity of 17000 K light source (Rosco Laboratories, Stamford, CT). Experimental illuminance/irradiance measures were taken using the IL-1400BL radiometer/photometer previously described which was held in place by a clear, plastic holder attached in an ophthalmologic head holder 30 cm from the panel at approximate eye level of the participants. Figure 2 shows the spectral power distributions emitted by the 4000 K and 17000 K experimental lamps.

Participants were randomly assigned on the day of entry to experimental polychromatic light from a 4000 K or a 17000 K fluorescent lamp (N=10 per condition). The target intensity of the 17000 K lamp was chosen from fluence-response curves for short-duration (90-minute) melatonin suppression testing [28]. The ED_{90}, or 90% of the saturating dose of light for melatonin suppression for the 17000 K lamp, was chosen for this study. This resulted in an equal photon density of 1×10^{14} photons/cm^2/s for both exposure conditions. The equivalent irradiance/illuminance of light for the 4000 K and 17000 K lamps were 40.7 μW/cm^2 (134.3 lux) and 50.0 μW/cm^2 (128.9 lux), respectively. The average irradiances (± SEM) measured every 30 minutes over the 6.5 h exposure were 40.86 ± 0.12 μW/cm^2 for the 4000 K exposure and 49.99 ± 0.07 μW/cm^2 for the 17000 K exposure. For 140 minutes prior to and for the full duration of the light exposure participants remained seated. Participants wore blindfolds for approximately 30 minutes prior to light exposure while lights stabilized and intensities were verified. Participants were seated 30 cm in front of the light panel with their chin placed in an ophthalmologic head holder and monitored continuously. Participants were asked to maintain a fixed gaze toward the panel for 90 minutes before resting their head and neck out of the headholder for 10 minutes while remaining seated. This sequence was repeated throughout the entire 6.5 h exposure.

2.4 Sleepiness and performance assessments

During every hour of wakefulness, participants completed the Karolinska Sleepiness Scale (KSS) [29], and visual and auditory psychomotor vigilance tasks (PVTs) [30,31] using a personal digital assistant (PDA) (Tungsten E2, Palm, Inc, Sunnyvale, CA). This modified visual PVT lasted for five minutes, at the end of which the mean reaction time

for that trial was displayed for the subject. PDA light measures taken at 12 inches from the eye were 1.2 lux and at 24 inches were 0.6 lux, The main measures for each trial for each subject were mean reaction time in milliseconds and number of lapses greater than 500 milliseconds (i.e. number of times the subject took 500 milliseconds or longer to react to the target appearing) [30,32,33]. The auditory PVT was completed on a PVT-192 by Ambulatory Monitoring, Inc. (Ardsley, NY). The auditory PVT trial was 10 minutes in length. The main measures for each trial for each subject were mean reaction time in milliseconds and number of lapses greater than 400 milliseconds [31].

2.5 Sample collection

Blood samples (3 ml) were collected through an indwelling intravenous catheter located in a forearm vein into 3 ml polystyrene tubes which contained 5.4 mg of K2 ethylenediaminetetraacetic acid (EDTA) (BD Diagnostics, Franklin Lakes, NJ). Plasma was separated by refrigerated centrifugation, aliquoted into cryogenic vials and stored at -20˚ C. Plasma melatonin samples were collected every 60 minutes during constant-routine periods on Days 3/5 and every 20 minutes during light exposure. Plasma melatonin concentrations were assayed by radioimmunoassay using antiserum described by Rollag and Niswender [34].

Saliva samples (2ml) were collected with the "Salivette" sampling device (Sarstedt, Inc., Hayward, CA). Specimens were centrifuged and aliquoted into cryogenic vials for storage at -20˚ C until assay. Salivary melatonin samples were collected every 60 minutes during constant-routine periods on Days 3/5 and during light exposure. Salivary melatonin

concentrations were assayed by radioimmunoassay [35]. Salivary analyses were used as a backup measure when plasma samples were missed due to IV line failure.

2.6 Melatonin assays

The inter-assay coefficient of variation from the 15 plasma assays run for this experiment using the assay from Rollag and Niswender [34] was 17.0%. Coefficient of variation calculated from a control sample of 100 pg/ml assayed had 12.3% for intra-assay coefficient of variation. The minimum detection limit of this assay is 0.5-2.0 pg/ml melatonin. The inter-assay coefficient of variation from the 7 salivary assays run for this experiment using the assay from Vaughan [35] was between 8-12%. Coefficient of variation calculated from control samples of 1.83 and 18.99 pg/ml assayed had 2.7% and 6.9% intra-assay coefficient of variation respectively. The minimum detection limit of the salivary assay is 0.5-2.0 pg/ml melatonin.

2.7   Statistics

Melatonin suppression (mean ± SD) was calculated from the difference in area under the curve (AUC) between melatonin profiles during light exposure and the corresponding clock times during the previous melatonin cycle on CR1 [16]. Unpaired, two tailed t-tests were used to compare differences in melatonin suppression by exposure to 17000 K lamps compared to 4000 K lamps.

$DLMO_{25}$ was calculated for each subject defined from 25% of the fitted three-harmonic peak-to-trough amplitude of the melatonin rhythm during the first constant routine. The magnitude of phase-shift was determined from differences in clock time between $DLMO_{25}$

during the first constant routine (CR1) as compared to the second constant routine (CR2) [16]. Unpaired, two tailed t-tests were used to compare differences in phase by exposure to 17000 K lamps compared to 4000 K lamps.

The data from the visual PVT and the auditory PVT-192 devices were analyzed using mixed ANOVA analysis comparing light sources and time was completed using SAS 9.0 (SAS, Cary, NC).

To determine sample size *a priori*, data from a previous study of the phase shifting effects of 460 nm and 555 nm monochromatic light were used to perform a power analysis [16]. Using these data, a 1-h phase shifting difference was assumed, with a standard deviation of 0.62 h, α value of 0.05, and β value of 0.9. With these assumptions, 10 participants per group were calculated to be needed to detect the 1 h phase difference [36].

3.0 Results

3.1 Melatonin suppression

As shown in Figure 3, all participants exposed to blue-enriched (17000 K) polychromatic fluorescent light had at least 29% melatonin suppression over the entire 6.5 h light exposure (range 29-91%). Suppression was more variable among participants exposed to the standard (4000 K) polychromatic fluorescent light at the same photon density (range 0-82%), including 2 individuals with virtually no melatonin suppression. On average, exposure to 6.5 h of 17000 K light caused a significantly greater suppression of melatonin (mean $\pm$SD, 70.9 $\pm$ 19.6%; n=10) compared with 4000 K light (42.8 $\pm$ 29.1%; n=10) ($p<0.05$). The time course of the melatonin suppression response also differed between the groups. In the 17000 K condition, the light suppressed melatonin for the entire exposure duration and did not return to DLMO levels. In the 4000 K condition, four participants

failed to suppress plasma melatonin to their $DLMO_{25}\%$ level at all and in four other participants, melatonin levels recovered to their $DLMO_{25}\%$ after ~1-3 hours within the light exposure. (1.35 h, 1.67 h, 2.50 h, 3.00 h) (Figure 4)

3.2 Phase shifting

Both light exposure conditions caused a phase delay of the onset of the melatonin rhythm in all but of the one of the participants. Exposure to 6.5 h of 17000 K light caused a phase delay shift with amean ± SDof 2.1 ± 0.6 h; n=10whileexposure to 4000 K light caused a phase delay shift of 1.7 ± 1.3 h; n=10. The mean differencedid not reach significance (p=0.22) (Figure 3). Representative 24 h melatonin profiles from an individual in each of the light conditions are shown in Figure 5.

3.3 Sleepiness assessment

As shown in Figure 6, self-rated sleepiness was lower during the 17000 K light exposure as compared to the 4000 K group (p<0.05). Pre light exposure differences in KSS were not significant (p=0.20). Participants in the 4000 K light group consistently reporting being sleepier at all time points (p<0.05) during LE compared to the 17000 K light group and remained so for 3 hours post light exposure (p<0.05).

3.4 Performance assessment

With the visual PVT testing, there was no significant difference between the two light groups in the mean visual reaction time (RT) or lapses of attention during, or for the three hours after, the light exposure ended.

Three participants were eliminated from the auditory PVT analysis for the 4000 K light group as their data files were corrupted due to equipment malfunction. For auditory RT, there were no significant differences in any parameter during the light exposure but there were significant differences between light conditions for RT and lapses > 400 msec for the 3 hours post LE (p<0.05), with the 17000 K light group having a quicker reaction time and fewer lapses than the 4000 K light group. This can be seen in Figure 6.
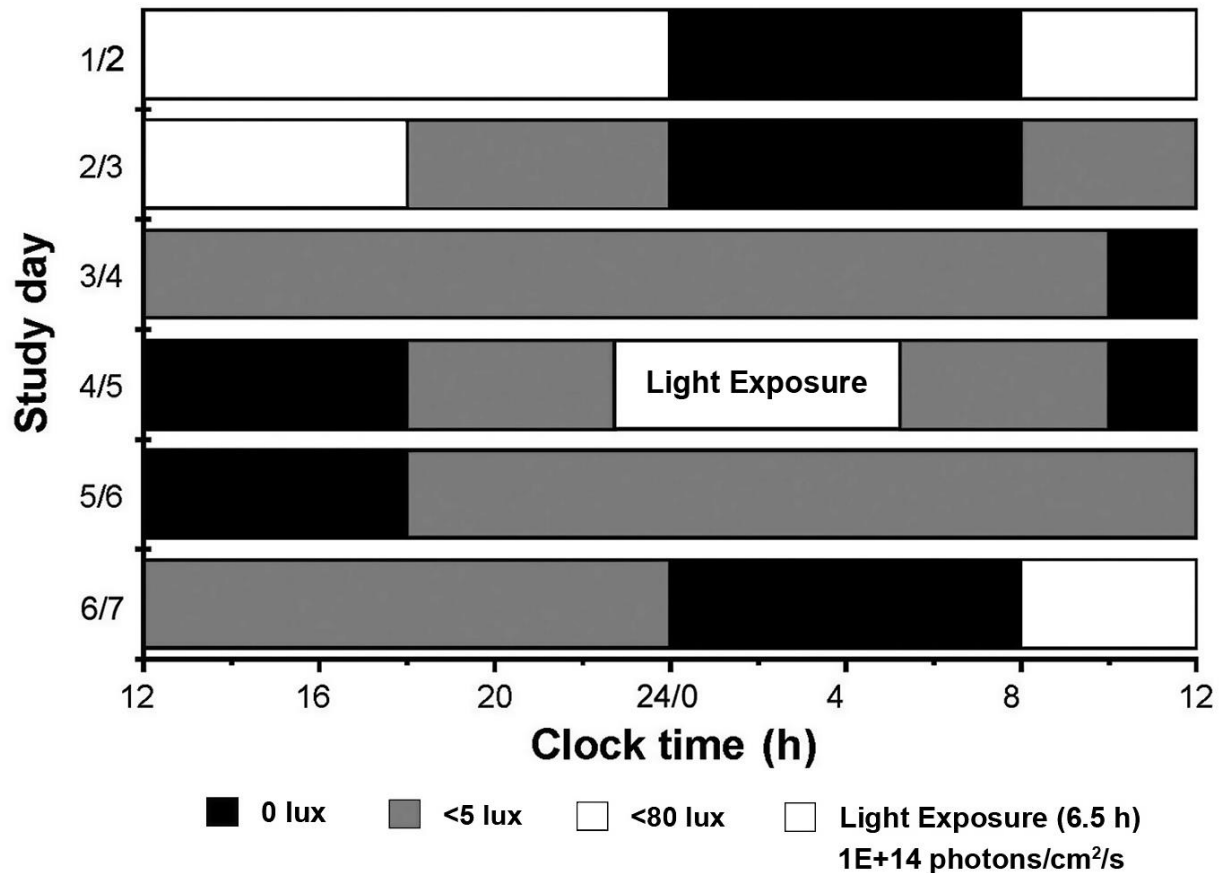
Fig. 1. Overview of 7-day phase shifting protocol for a subject with a midnight to eight AM sleep:wake schedule. The schedule consisted of a 2-day baseline (8-hour:16-hour sleep-wake cycle based on each subject's self-selected sleep-wake times), an initial 26 hour constant routine, a 16 hour dim light-exposure day with a 6.5 hour experimental light exposure, and a second 30 hour constant routine, each preceded and followed by an 8-hour sleep opportunity. [16,19].
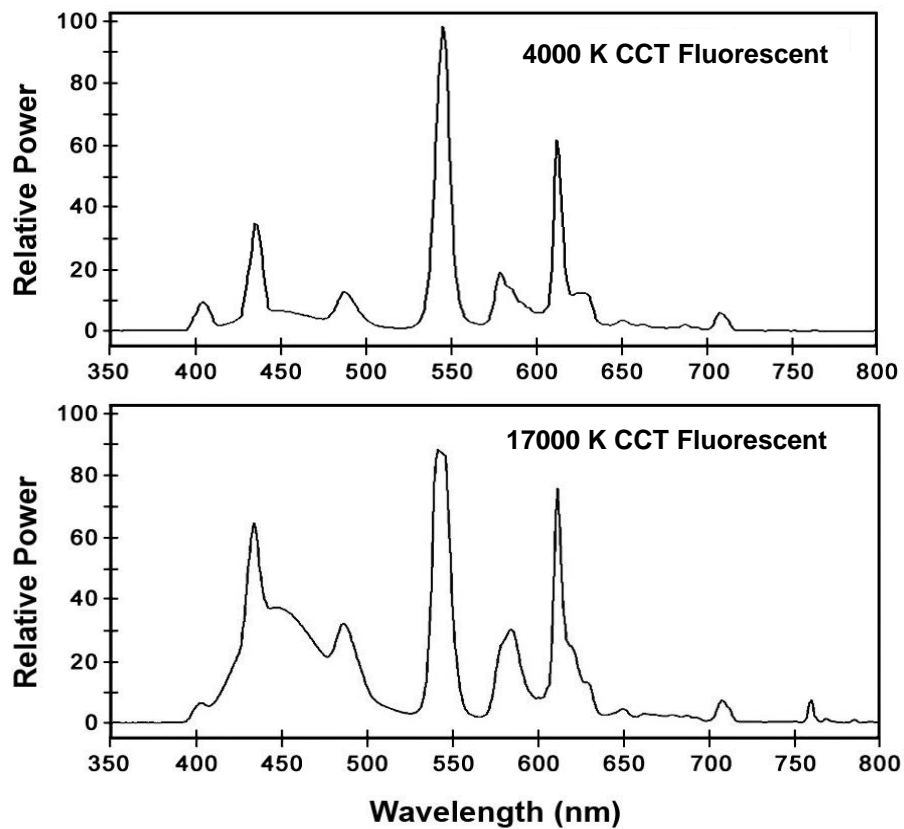
Fig. 2. Spectral power distribution for 4000 K (upper panel) and 17000 K (middle panel) light sources. Profiles are relatively similar, with the primary difference between 400 and 500 nm. Compared to the 4000 K light source, the 17000 K light source emitted substantially more power in the blue light part of the spectrum. The lower panel shows calculated irradiances, photopic illuminances $v(\lambda)$, and human photopigment illuminances relative to the two polychromatic light sources used [75].
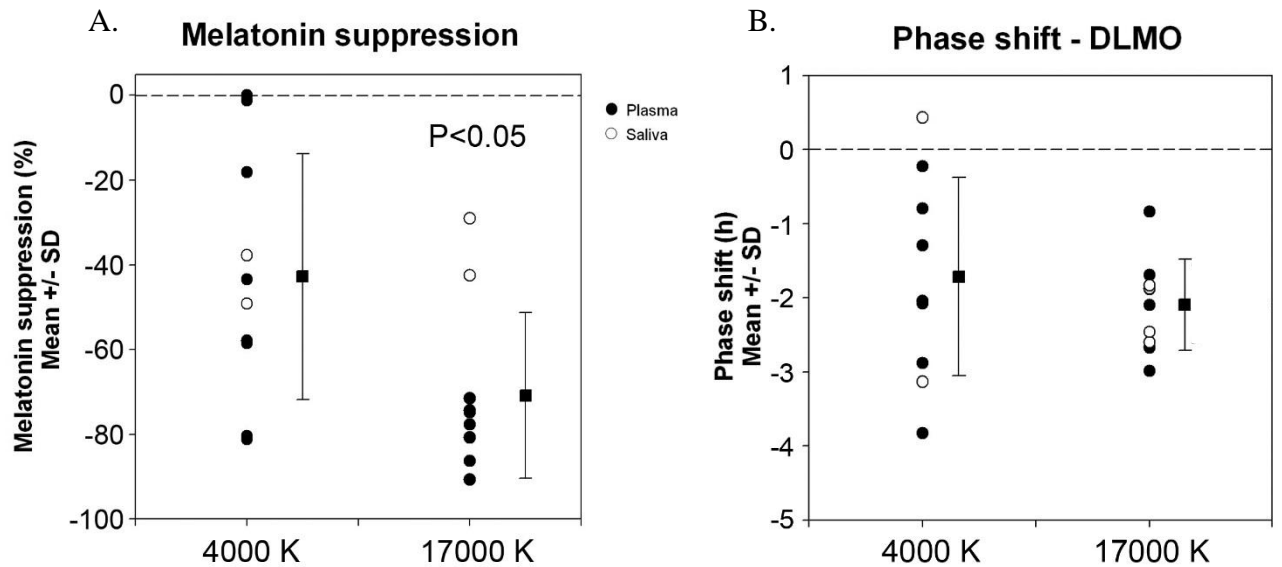
Fig. 3. Melatonin suppression (±SD) as calculated by AUC with plasma (filled circles) or salivary (empty circles) levels following exposure to 6.5 h of white 4000 K fluorescent light or blue-enriched 17000 K fluorescent light (A). A greater suppression of plasma melatonin occurred under 17000 K fluorescent light compared to 4000 K fluorescent light (p<0.05). Phase delay shift (±SD) of the plasma (filled circles) or salivary (empty circles) melatonin rhythm as assessed by DLMO following exposure to 6.5 h of white 4000 K fluorescent light and blue-enriched 17000 K fluorescent light (B). The phase delay shift, was not statistically significant (p=0.22).
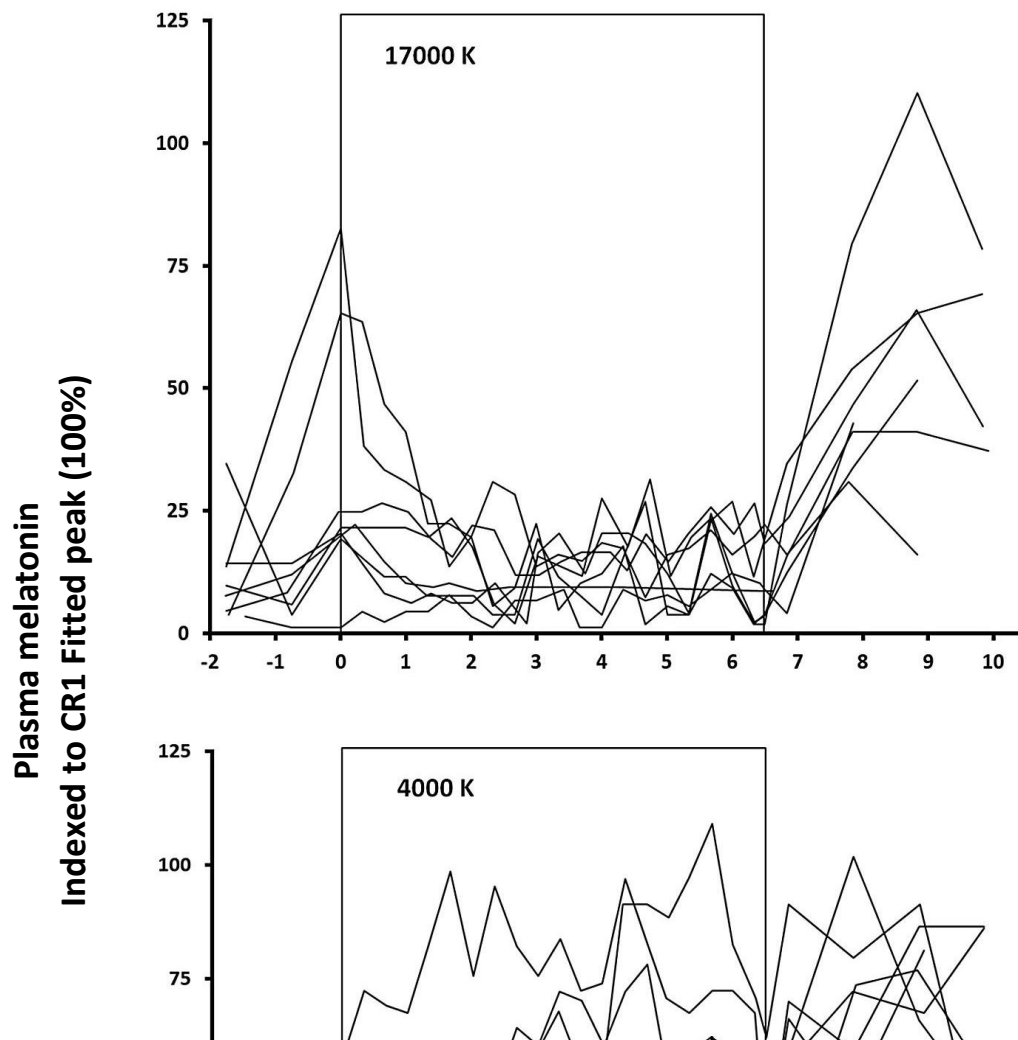
Time since lights on (h)

Fig. 4. Individual melatonin profiles 2 h prior to, during (boxed area) and 4 h after 6.5 h exposure to 17000 K (top panel) and 4000 K (bottom panels) light, normalized to each individuals' fitted peak value during the first melatonin cycle on CR1.
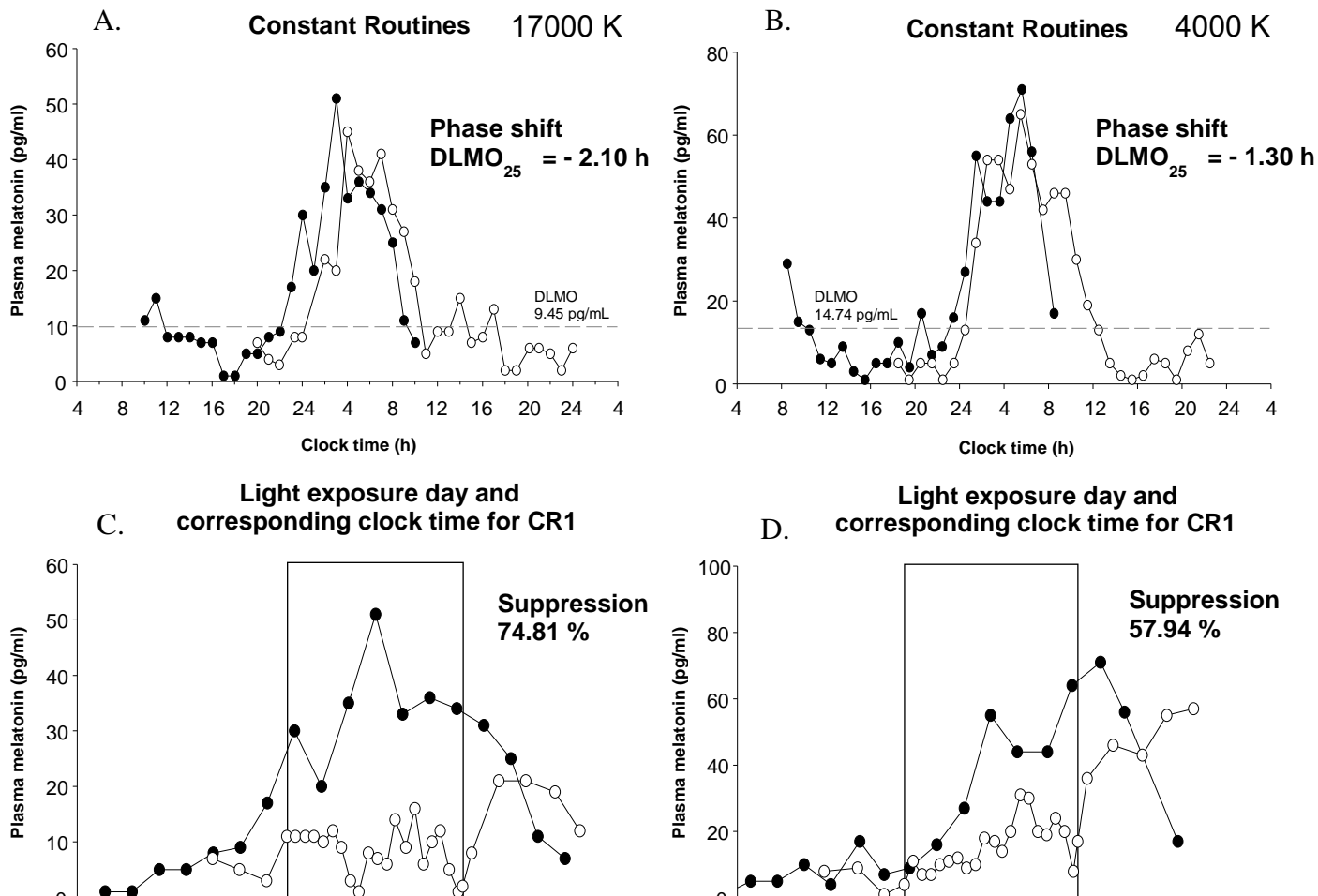
Fig. 5. The 24 h melatonin profiles on CR1 (filled circles) and CR2 (open circles) from one representative individual in each of the LE groups (A & B). Below each (C & D) are the calculated melatonin suppression (AUC) during LE (open circles) and the corresponding time from CR1 (filled circles) for each of the same individuals.
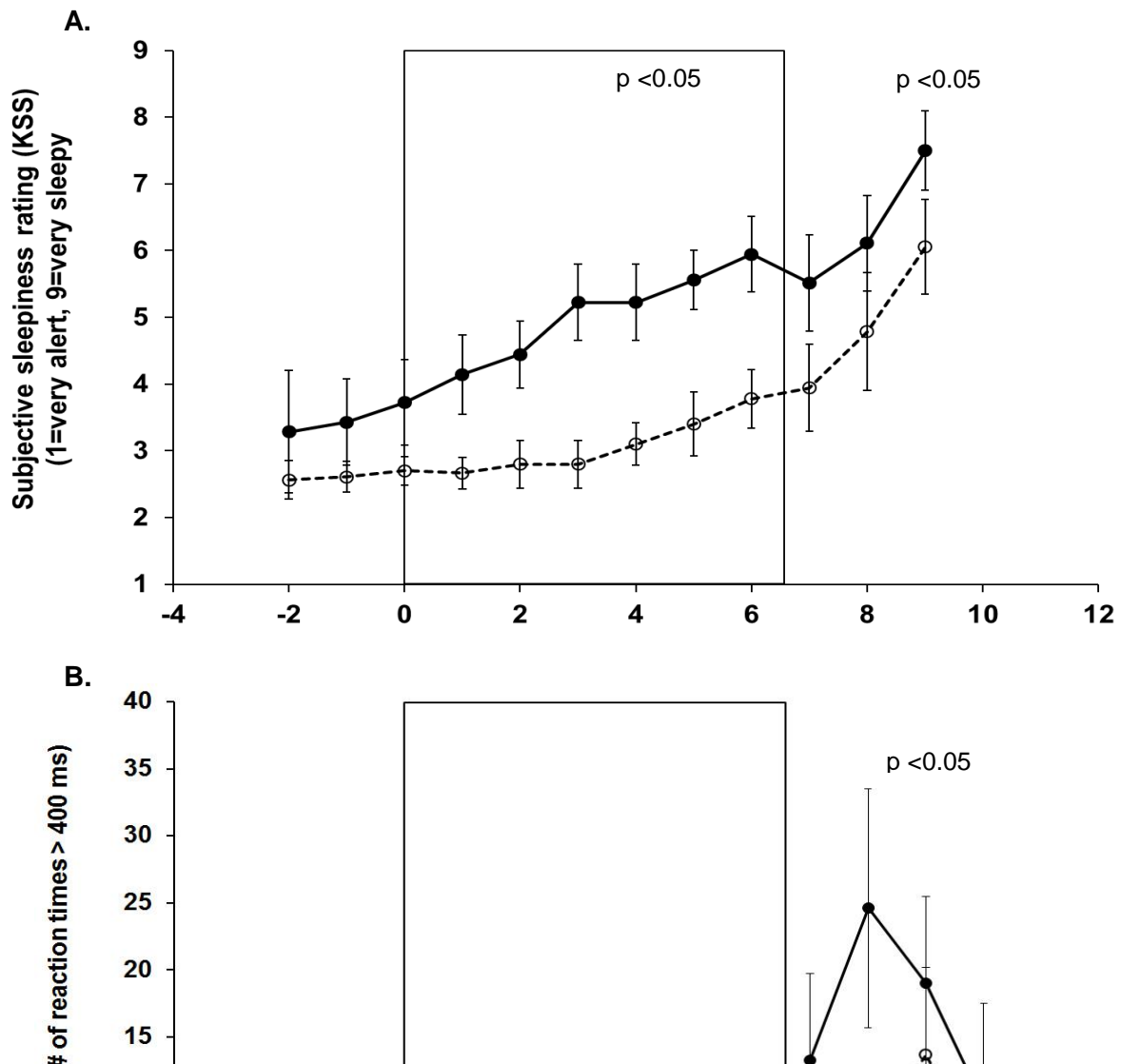
Fig. 6. KSS scores before, during and after LE (filled circles 4000 K; open circles 17000 K, top panel). KSS scores were lower, indicating higher alertness, during LE for the 17000 K group versus the 4000 K group (p<0.05). Average auditory PVT lapses per hour before, during, and after LE. The period of LE is indicated by box (bottom panel)

4. Discussion

Our results demonstrate that both standard (4000 K) and blue-enriched (17000 K) fluorescent light at equal photon densities of 1 x 10^{14} photons/cm^2/s suppress plasma melatonin in healthy young participants with blue-enriched light eliciting a stronger hormonal suppression. In contrast, phase shifting was not significantly different at this intensity between the two light conditions. Subjective measures of alertness using KSS support the hypothesis that blue-enriched light exposure significantly improves alertness. Objective measures of alertness using PVT, however, did not show any significant differences in alertness during the light exposures. In the three hours post-light exposure

the blue-enriched light group had quicker reaction times and fewer lapses compared to the standard light group.

This study was based on two dose-response curves for melatonin suppression which predicted the photon density at which standard and blue-enriched light would differ the most. The ED_{90} for the 17000 K dose-response curve with its matching 4000 K dose (approximately ED_{50}) was used [28]. Use of these two dose-response curves successfully predicted the resultant melatonin suppression observed here.. The blue-enriched fluorescent light provided a significantly more consistent phase-delay than standard fluorescent light when comparing variances, however, indicating a greater effect on the circadian system. Our pre-study power calculations based on monochromatic exposure data showed sufficient power to detect an effect on phase shifting but our post-hoc power calculation revealed that these data yield a β value of 0.797, at α value of 0.05. This is a statistical power level (1-β) of 20.3%. In order to obtain a statistical power of 50%, i.e. β=0.5, each group would need to have 41 subjects, for a total of 82 subjects in the study, based upon the mean difference found between the 17000 K and 4000 K groups. The costs of running such a study would be prohibitive and is an obvious limitation to this study.

Similarly, the blue-enriched condition also had a more sustained response on melatonin suppression. When examining the time course of melatonin suppression over the 6.5 hour light exposure, the blue-enriched light was effective for the entire 6.5 hours whereas the standard lighting either failed to cause suppression or showed a loss of sensitivity over time, with melatonin returning to its DLMO level after an average of 2.15 hours. This is consistent with our previous finding that longer-wavelength monochromatic light cannot

sustain melatonin suppression responses for more than a few hours [16,19], presumably through the inability of cone photoreceptors to maintain a maximal response [19,37]. When attempting to differentiate the relative contribution of photoreceptor systems it is useful to assess the light response over long durations in order to detect time course differences.

How classic photoreceptors participate in this phototransduction process remains an area of intensive investigation. Different types of ipRGCs with distinct retinal connections [38] further complicate the picture as perhaps these differing classes combine rod and cone inputs differentially leading to changing spectral sensitivities for the different evoked light responses. One should never assume that a light response in one area of physiology such as a neuroendocrine response like acute melatonin suppression will be predictive of another studied response such as phase shifting the circadian pacemaker. The data presented here illustrate that point.

Objective and subjective measures of alertness yielded inconsistent results. While blue-enriched light exposure improved subjective alertness both during and after the light exposure compared to standard lighting, performance improvements were only seen in the three hours after the light exposure, and only for auditory reaction time and lapses. While it is not unusual for subjective and objective measures of alertness to show inconsistent changes with sleep deprivation [39,40] or light exposure [41,42], further work is required to understand the source of these differences, including the role of visual perception in mediating subjective responses.

A number of other comparative polychromatic light studies have also failed to find differences in phase shifting responses [43,44,45]. Smith and colleagues did not find

differences in phase delay or phase advance shifts but used very high, potentially saturating light levels (4000-6000 lux, ~4.2 x 10^{15} photons/cm^2/s). Munch and colleagues used a lower intensity (1 x 10^{15} photons/cm^2/s) closer to, but still an order of magnitude higher than that used in the current study (1 x 10^{14} photons/cm^2/s), and studied older adults who were likely to have reduced sensitivity to blue-light given pigmentation of the lens that would have blocked some of the short-wavelength light [46]. While we used a quantitative approach in selecting our light intensity, basing it on short-duration fluence response curves for melatonin suppression using the same light sources, it is possible that i) short duration exposures (1.5 h) do not always predict the effects of longer duration light (6.5 h) [19]; ii) the magnitude of melatonin suppression does not predict the magnitude of circadian phase shifting or vice versa [47,48]. Our pre-study power calculations based on monochromatic exposure data showed sufficient power to detect an effect on phase shifting but our post-hoc power calculation for phase shifting, based on the actual variances measured, suggested that a greater number of participants was required.

The blue enriched light source used appears to be more efficient for photic phase shifting than the standard light source . Results were compared with a prior white light PRC [8] and indicated that the blue enriched light stimulus achieved approximately 80% of the phase shifting response of the much brighter (3,000 µW/cm^2) white light stimulus with the maximum phase-delay of -2.6 h while the standard light stimulus achieved 25% less. In our study, we used less than 2% (50 µW/cm^2) of the energy of the white light stimulus reported in the PRC study.

The melatonin suppression data presented here are similar to the melatonin suppression results of other studies comparing 4000 K and 17000 K fluorescent lights. Specifically,

compared to the 4000 K light, 17000 K light was significantly stronger in suppressing melatonin in healthy subjects [28,49]. Melatonin suppression has been a widely used marker to discern physiology of melatonin and circadian regulation [50]. Importantly, melatonin suppression by light is suspected to be oncogenic [51] as well as possibly contributing to a range of other disorders [52]. Strict numerical comparison of melatonin suppression values across these studies, however, is not warranted due to major protocol differences such as circadian time of light exposure, prior photic history, light exposure duration and light source geometry [8,53,54,55,56].

Many studies, but not all [57,58], have shown that polychromatic light increases alertness and improves performance in neurobehavioral tests compared to dim light or darkness. The study reported here uses equal photon doses of relatively dim light. A dark control condition may have yielded significant results. Studies using polychromatic light reported alerting effects when used at night [59,60,61,62]. Neurobehavioral and physiological parameters affected by nocturnal bright light exposure include subjective sleepiness, EEG frequencies in the beta, theta and alpha ranges, psychomotor vigilance performance, and a variety of cognitive tests. These effects have also been shown for daytime exposure [63,64] consistent with a separation of the mechanisms underpinning the alertness-enhancing and melatonin suppression responses of light, at least during the day.

When comparing the alerting effects of light with different spectra, a less consistent view emerges. In controlled laboratory studies of monochromatic, narrow-band light and polychromatic light, short-wavelength sensitivity to the alerting effects of light on subjective and objective measures have been shown in a range of models and different times of day [65,66,67]. Similarly, the benefits of blue-enriched daytime light exposure on

alertness, performance, mood and cognition have been seen in real-world settings such as schools, offices and care homes [23,24,25,26,27,68] and in clinical groups [69,70]. Other studies, however, have failed to show major differences in alertness and/or performance measures between different narrow-band or polychromatic sources during the day [39,71] or night [41].

Major differences in populations studied, parameters measured, light history experienced, timing of light exposure given and intensities employed all likely contribute to these findings. While there is likely too much variability between studies to conduct a formal review of the alerting effects of light at this time, the weight of evidence suggests that blue or blue-enriched lighting is likely to be beneficial for alertness and performance compared to spectra with less short-wavelength light, all else being equal.

Modeling these effects of light, or designing lighting to take advantage of these benefits in real world application, presents a challenge. There are multiple factors that affect these responses to light, including circadian timing, light intensity, wavelength and duration, but the field does not have a complete knowledge of all relevant factors behind such effects. While models have been proposed to try and predict the relative efficacy of a particular light stimulus in terms of its relative circadian, neuroendocrine, and neurobehavioral efficacy [72,73,74] caution must be exercised as our understanding of circadian phototransduction develops.

Lack of a consistent and adequate method of quantifying light between different laboratories publishing data on photic regulation of biological and behavioral responses can make it challenging to replicate experimental conditions or to compare across studies. A number of laboratories have collaborated on developing a consensus publication about

measuring light for circadian, neuroendocrine and neurobehavioral regulation based on current and accepted sensitivity functions for rods, cones and melanopsin integrating the spectral power distribution of the source [75] and the Commission Internationale de l'Eclairage recently adopted the consensus publication and accompanying free Toolbox as an international standard [76].   Light source data are reported in the Figure 2 using the Toolbox described by Lucas and colleagues [75]. While quantification of the biological potency of light for non-visual photoreception is developing, collecting source descriptions on one platform will help for comparing results and developing testable hypotheses that predict spectral characteristics for targeted physiological responses to polychromatic light. Care must be taken to appropriately describe and measure the lighting conditions so as to inform the lighting community and foster intelligent design. Careful measurement of the incident light spectrum being studied is crucial and must be reported in a systematic way. To this end, the consensus methodology described by Lucas and colleagues [75] with use of a common nomenclature based on current and accepted sensitivity functions will open the door for researchers to continue to unravel this complex photosensory system. Meanwhile, empirical verification of specific light stimuli will remain the most accurate way to determine the biological potency of light sources.

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Competing Interest

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