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Changing color and intensity of LED lighting across the day impacts on circadian melatonin rhythms and sleep in healthy men

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Abstract

We examined whether dynamically changing light across a scheduled 16-h waking day influences sleepiness, cognitive performance, visual comfort, melatonin secretion, and sleep under controlled laboratory conditions in healthy men. Fourteen participants underwent a 49-h laboratory protocol in a repeated-measures study design. They spent the first 5 hours in the evening under standard lighting, followed by an 8-h nocturnal sleep episode at habitual bedtimes. Thereafter, volunteers either woke up to static light or to a dynamic light that changed spectrum and intensity across the scheduled 16-h waking day. Following an 8-h nocturnal sleep episode, the volunteers spent another 11 hours either under static or dynamic light. Static light attenuated the evening rise in melatonin levels more compared to dynamic light as indexed by a significant reduction in the melatonin AUC prior to bedtime during static light only. Participants felt less vigilant in the evening during dynamic light. After dynamic light, sleep latency was significantly shorter in both the baseline and treatment night while sleep structure, sleep quality, cognitive performance, and visual comfort did not significantly differ. The study shows that dynamic changes in spectrum and intensity of light promote melatonin secretion and sleep initiation in healthy men.

KEYWORDS

cognition, humans, Lighting, male, melatonin, nonvisual effects of light, sleep, wakefulness

1 | INTRODUCTION

In our modern societies, we spend increasingly more time indoors under electric light.[1] As a consequence, we expose ourselves to more electric light and less sunlight during the day. Exposure to electric light after sunset can potentially delay our internal clock with the risk of desynchronizing circadian rhythms from sleep-wake rhythms. In order to avoid such circadian misalignments, it is recommended[2] to increase Zeitgeber (ie "time giver") strength by increasing light exposure during the day and avoid light at night. The wrong light at the wrong time of day is also associated with negative effects on well-being and sleep.[3-6]

Intrinsically photosensitive retinal ganglion cells (ipRGCs) use the photopigment melanopsin to transmit light stimuli via the retinohypothalamic tract into different brain areas, primarily to our internal "master clock," the nucleus suprachiasmaticus.[7-10] The photopigment melanopsin is very sensitive to short wavelength light, that is blue appearing light, making light of these wavelengths highly effective in their function as a Zeitgeber.[11,12] Especially blue and white appearing light with a high proportion of short-wave radiation during the evening and at night attenuates the natural increase in sleepiness and in melatonin secretion.[13,14] The production of melatonin can be acutely inhibited by light.[15,16] Two in-depth investigations with monochromatic light ranging from 420 to 600 nm found wavelength regions between 457-462 nm[13] and 446-477 nm[14] as being most powerful for melatonin suppression.

Effects of a simulated dawn on circadian rhythms in humans were investigated by Danilenko et al[17] Control participants remained under dim light conditions with an alternating light-dark cycle (< 30:0 lx). Their results showed that a replacement of the last 1.5 hours of darkness by a natural dawn stimulus was sufficient to maintain an entrained phase position in comparison with the control situation. In a study in which both the correlated color temperature (CCT) (1090-2750 K) and illuminance at the eye (0-250 lx) was changed during wake up in the morning, mood, well-being, and cognitive performance increased,[18,19] and the cardiac control during the awakening process was better[20] in comparison with 8 lx. A twilight simulation was also found to be antidepressant[21] and to eliminate poor sleep patterns.[22] In a study comparing dynamic light with static light for office workers no positive effects on sleep, vitality and productivity of dynamic light were found. The employees were subjectively more satisfied with the dynamic light than with the static light, however.[23] To date, nonvisual light effects were predominantly studied during the evening or at night, and to a lesser extent during the day. Twilight studies and the combination of the three time points (ie night, day, and twilight) investigating the continuous change of light during 16 hours of wakefulness are rare, and therefore, it remains unclear if these changes improve circadian physiology, visual comfort, cognitive performance, and sleep.

In the present study, we compared a static lighting condition "sLED" with a dynamically changing light "dynLED" that incrementally increased both CCT and illuminance in the morning and continuously decreased both in the afternoon until bedtime. This light profile is generally assumed to have various positive effects on humans, although this has not yet been tested rigorously. We expected less melatonin suppression in the evening prior to bedtime and better sleep, indexed by more EEG delta activity in the treatment night, after the dynamic compared to the static light condition. Furthermore, we expected better visual comfort, alertness, and cognitive performance during the 16 hours of wakefulness during dynamic compared to the static light conditions, particularly during times when exposed to 5000 K compared to static light at 4000 K. In the evening, however, we expected better cognitive performance and higher alertness during static light due to both higher intensity and higher CCT than during the dynamic light.

2 | MATERIALS AND METHODS

The design and method of the present study was based on our previously published study[24] and is thus summarized here. The study procedures were approved by the local ethics committee and performed according to the Declaration of Helsinki. All participants provided written informed consent.

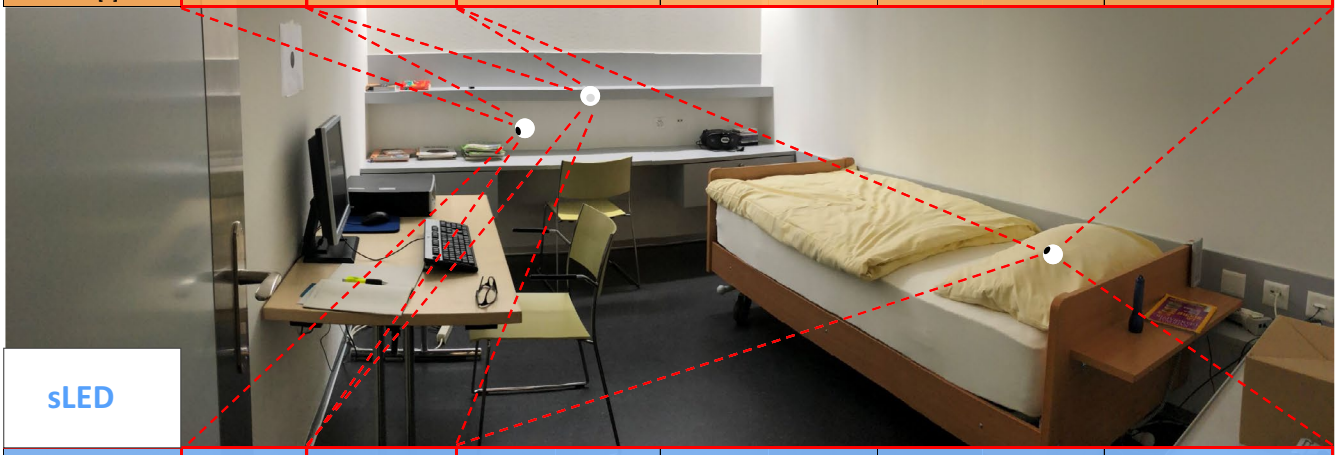
2.1 | Study design

The "in-laboratory part of the study" comprised two 49-h episodes, which participants spent in sound-attenuated suites under light, temperature, and humidity controlled conditions without time cues (Figure 1).[24] All study events were timed relative to each individual`s habitual sleep-wake times. Volunteers reported to the laboratory 6 hours prior to their usual bedtime and spent the first evening under standard fluorescent lighting conditions (Philips Master TL5 HO 54W/830, CRI 80, 3000 K) with a horizontal illuminance of 88 lx (29.5 lx melEDI) on the pillow. After an 8-hour baseline sleep episode, volunteers either woke up in the sLED or dynLED condition (Toshiba TRI-R Circadian System NP10576, based on TRI-R LED SMD5056) and spent 16-h awake, followed by a second 8-h sleep episode (ie treatment night) and a final 11-h episode of scheduled wakefulness (Figure 2A). The order of the lighting conditions was counterbalanced and pseudo-randomized, such that half of the participants started with sLED and vice versa. Participants were under the same conditions in both nights (baseline and treatment). During scheduled wakefulness, volunteers were allowed to move freely in their room when they were not involved in scheduled tasks. They received the same scheduled meals (25 min, 4, and 11 hours after wake up). The washout period between the two in-laboratory sessions was one week.[24]

2.2 | Participants

Eighteen healthy male participants were screened for sleep and psychiatric disorders and spent one habituation night in the sleep lab prior to study participation. This habituation night, scheduled according to their habitual bedtimes, included a polysomnography screening, in order to assess

dynLED	2.5 – 7.5 hours after wake up				Wake up light		10 min before sleep		1 hour before sleep	
	Sitting at desk eye hight 120 cm vertical viewing direction -15°	Sitting at wall eye hight 120 cm vertical viewing direction -15°	Lying in bed height 75 cm vertical	Lying in bed hight 75 cm horizontal	Lying in bed hight 75 cm horizontal	Lying in bed hight 75 cm vertical	Lying in bed hight 75 cm horizontal	Lying in bed hight 75 cm vertical	Lying in bed hight 75 cm horizontal	Lying in bed hight 75 cm vertical
Illuminance [lx]	83	273	110	96	1.6	1.4	1.1	1.1	12.5	14.2
Irradiance [µW/cm²]	31.6	107.1	41.8	36.1	0.6	0.5	0.4	0.4	5.0	5.6
CCT [K]	4528	5080	4746	4619	3652	3725	2544	2585	2466	2486



sLED										
Illuminance [lx]	87	299	129	111	129	111	129	111	129	111
Irradiance [µW/cm²]	33.1	116.4	49.2	42.0	49.2	42.0	49.2	42.0	49.2	42.0
CCT [K]	3498	3802	3658	3584	3658	3584	3658	3584	3658	3584

FIGURE 1 Lighting parameters during the study at different times (only for the dynamic condition) and at different locations in the laboratory for both conditions

their ability to sleep in a new environment and to rule out sleep disturbances. They spent the habituation night one to three weeks prior to the in-laboratory part of the study. All participants had a good sleep quality as assessed with the Pittsburgh Sleep Quality Index[25] (PSQI score ≤ 5) and were no extreme chronotypes (42 and 57 points on the Munich Chronotype questionnaire[26]). They underwent a medical examination carried out by a study physician and an ophthalmic examination by a certified optometrist to exclude volunteers with visual impairments. All participants were screened for color deficiency by the Ishihara Test.[27] Exclusion criteria were smoking, medication or drug consumption, shift work within the last three months, and transmeridian flights up to one month prior to the study. One week prior to the laboratory admission, participants were instructed to keep a regular sleep-wake schedule with a sleep duration of 8 hours, which was verified by sleep logs and continuous wrist actimetry. Actimetry-derived sleep duration was on average 8 hours and 8 minutes (SD: 28 min) during the week prior to the in-laboratory part of the study, with an average sleep start time at midnight (00:03). The range was between 7:25 and 9:00 hour. During the washout period, participants were instructed to keep the same regular sleep-wake schedule as one week prior to the lab visit (no naps, ±60 min within habitual bed times). Participants were asked to refrain from alcohol, and a toxicological screen was performed upon laboratory entry. After dropouts due to headache (N = 1), and technical issues (N = 3), data of 14 participants (mean age 25.58 ± 3.34 years) entered statistical analysis.

2.3 | Light treatment

During scheduled wakefulness, light exposure in the study room during sLED was set to 4000 K and 87 lx (vertical at the eye; Figure 1). Horizontal illuminance at desk height ranged, depending on the position, between 150 and 650 lx, which corresponds to standard office lighting. DynLED was defined as a continuous light change starting in the morning with 3500 K/<1 lx incrementally increasing until reaching a peak of 5000 K/83 lx 2.5 hours after wake up lasting until 7.5 hours after wake up. The lighting conditions are illustrated in Figure 1 and Figure 2. Afterward, CCT and illuminance slowly and continuously decreased in the afternoon reaching 2700 K/<1 lx at bedtime. During dynLED, the luminance 10 min prior to sleep on the pillow was 0.3 cd/m^2. Horizontal irradiances on the pillow at that time during sLED were 100-fold higher than during dynLED (42 vs. 0.43 µW/cm^2). Lighting transitions did not occur at fixed clock times but varied for each individual relative to their habitual sleep-wake schedules. For easier readability, we refer to the example of bedtime at midnight in Figure 2A. This corresponds to the average habitual bedtime of the participants. Responses of all photoreceptors

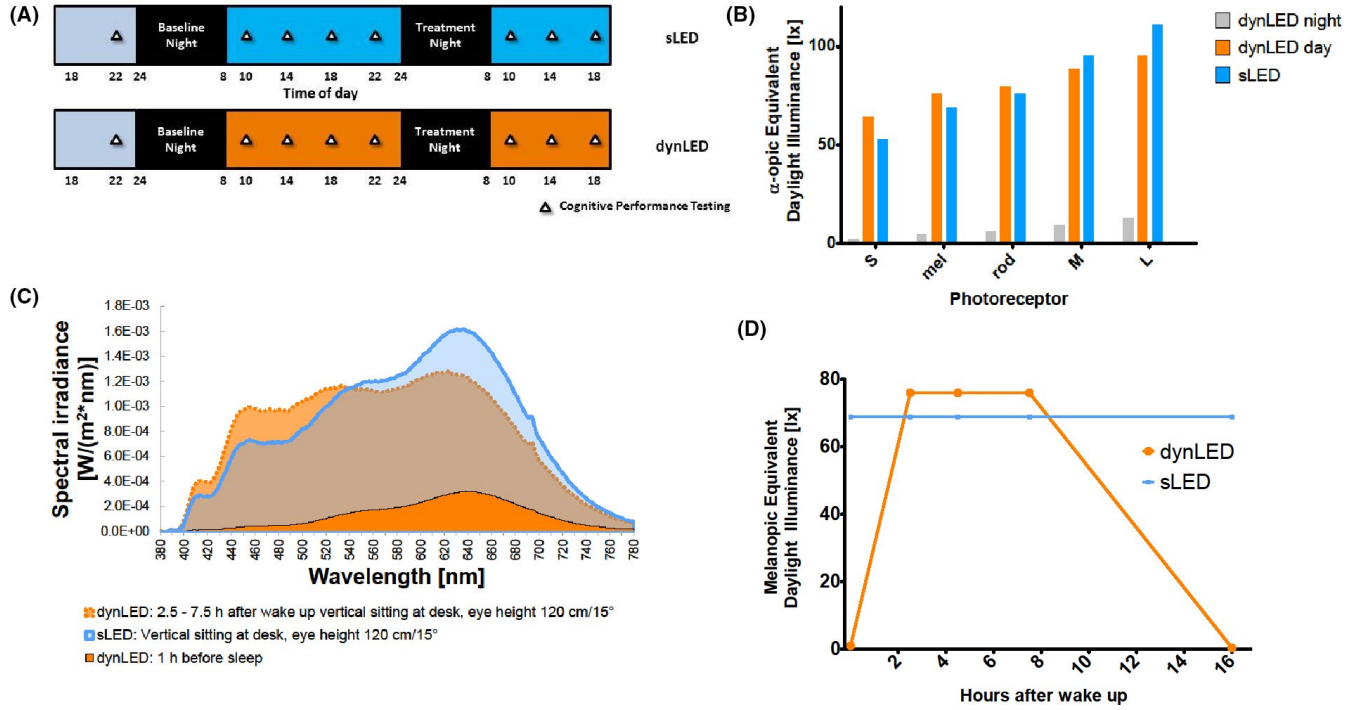


FIGURE 2 A) Schedule of the study: Participants spent twice 49 h in the laboratory, once under a static LED light condition (sLED in blue) and once under dynamically changing LED light (dynLED in orange). The first evening (gray), they spent in identical conditions. Triangles show the timing of cognitive performance testing. Salivary melatonin samples were taken half hourly in the evening and every two hours during the day. B) Responses of all photoreceptors according to the new CIE standard.[28] Depicted are EDIs for the five photoreceptors: S-cones, melanopsin, rods, M-cones, and L-cones during the day for sLED and dynLED and one hour prior to sleep only during dynLED. C) Light spectra used during the study: Spectral irradiance vertical at eye height (120 cm −15°) sitting at the desk during dynLED (between 2.5 and 7.5 h after wake up) and sLED (at all times). The lowest curve shows the spectral irradiance during dynLED one hour prior to bedtime. D) Change of melanopic EDI [lx] during the study at different times of day. Between 2.5 AM and 7.5 h after wake up, melanopic irradiance is higher in the dynLED condition compared to sLED. After 7.5 h after wake up and especially in the late evening, melanopic EDI is significantly lower in the dynLED condition compared to sLED

according to the new CIE S 026/E:2018 standard[28] are shown in Figure 2B. According to this standard, we also report light intensities as melanopic equivalent daylight illuminance (melEDI) in lx. Ten minutes prior to sleep, melEDI during dynLED was 0.42 vs. 68.9 lx for sLED. One hour prior to sleep, melEDI was 4.5 lx during dynLED. Spectral characteristics are shown in Figure 2C. Figure 2D shows the change of melanopic EDIs across the day.

2.4 | Cognitive Performance and Subjective Variables

During the 16 hours of scheduled wakefulness, cognitive performance was assessed in four hourly intervals. Participants performed all tests in front of a black computer screen. Among various subjective variables, the 35-min cognitive test battery included a visual verbal n-back task and a psychomotor vigilance task (PVT). The PVT was presented on a gray background resulting in an illuminance of 9.3 lx at a viewing distance of 70 cm (6.8 lx melEDI). During the n-back task, volunteers indicated whether a displayed letter matched a target stimulus that was presented n trials ago. Each session consisted of six blocks, divided into two bouts. The demand level was adjusted to individual performance levels of the volunteers. For further information, please refer to.[24]

The PVT is a sustained attention task that is sensitive to circadian rhythmicity and sleep need.[29] Volunteers were requested to press a response button as fast as possible as soon as they heard an auditory stimulus while trying to avoid pressing too soon. The task lasted 10 minutes during which the stimulus was presented in intervals randomly varying from 2 to 9 seconds. The participants were able to familiarize themselves with the n-back task and the PVT during the baseline evening to wash out any learning effects.

During the entire laboratory stay, volunteers periodically rated their sleepiness levels on the Karolinska Sleepiness Scale (KSS)[30] in hourly or two hourly intervals. To assess the volunteer's subjective perception of visual comfort, we used a five-point Likert scale that probed brightness, and CCT based on a selection of questions derived from Eklund and Boyce.[31]

2.5 | Melatonin and PSG

Saliva collections were scheduled every 30 minutes in the morning and evening and every 1 or 2 hours in-between. A direct double-antibody radioimmunoassay was used for the melatonin assay (validated by GC-MS with an analytical least detectable dose of 0.65 pg/mL; BÜHLMANN Laboratories AG, Direct Saliva Melatonin RIA (RK-DSM2), Schönenbuch, Switzerland). The minimum detectable dose of melatonin (analytical sensitivity) was determined to be 2 pg/mL, and the interassay coefficients of variation (CVs) were 20.1% at 0.60 pg/mL, 2.6% at 7.24 pg/mL, and 4.8% at 24.42 pg/mL. The assay was performed by a third party, (NovoLytiX GmbH) according to the Instructions of Use of RK-DSM2, Version 2012-11-20.[32]

Melatonin onset was calculated for both evenings. We fitted the evening melatonin profile by a piecewise linear-parabolic function using the interactive computer-based hockey-stick algorithm to calculate the individual melatonin onset.[33] The AUC (area under the curve) of melatonin was calculated by approximating the integral of the last 10 melatonin values (4.5 hours prior to bedtime) using the trapezoidal method.

Sleep EEG activity was continuously recorded with the Vitaport Ambulatory system (Vitaport-3 digital recorder TEMEC Instruments BV, Kerkrade, the Netherlands). Twelve EEG derivations referenced against linked mastoids, two electrooculograms, one submental electromyogram, and one electrocardiogram were recorded. All signals were low pass filtered at 30 Hz at a time constant of 1 Hz. Sleep stages were visually scored per 30-s epochs according to standard criteria of the AASM.[34] Nonrapid eye movement sleep (NREMS) was defined as the sum of N2 and N3. Spectral analysis was conducted using a fast Fourier transformation, which produced a 0.25 Hz bin resolution. EEG power spectra were calculated during NREMS in the frequency range from 0 to 32 Hz. Artifact-free 4-s epochs were averaged across 30-s epochs. Here, we report EEG data from frontal (Fz, F3, F4) derivations, in the frequency range between 0.75 and 20 Hz.

2.6 | Statistical analysis

Statistical analyses were performed using SAS (version 9.4; SAS Institute). An alpha level of 0.05 was used to assess statistical significance. All output variables were analyzed with mixed-model analyses of variance (PROC MIXED) with the main repeated factors "light" and "time of day" and volunteer as random factor. Since reaction times and lapses of the PVT were not normally distributed, we analyzed the results using GLMM [Generalized Linear Mixed Models (PROC GLIMIX)]. For PVT performance, the default performance metrics – median reaction time (RT), 10% fastest and 10% slowest RT and lapses were calculated according to Blatter et al.[29] Response times below 100 ms were considered as false starts and excluded. For n-back performance, the following metrics were used: number of hits, false alarms, accuracy, and the percentage of correct responses. Since sleep latencies were not normally distributed, we log-transformed them prior to the analysis. All-night EEG power density in NREMS was analyzed for frontal derivations for each 0.25 Hz frequency bin, with the main factor "light." NREM-REMS cycles were defined according to an adapted method from Feinberg and Floyd.[35] Thereof, each sleep cycle was subdivided into ten time intervals of equal length during NREMS and into four time intervals during REMS episodes.

3 | RESULTS

3.1 | Cognitive performance

3.1.1 | PVT

The time course of PVT performance was rather stable across the day during both light conditions and did not reveal a significant main effect of the factor "light" for the different measures. Also, the factor "time of day" and the interaction "light" x "time of day" did not yield any significant effects, neither for median RT, the 10% slowest or 10% fastest RTs nor the attentional lapses.

3.1.2 | N-back task

There was no effect of the factor "light." The factor "time of day" yielded significance with better performance in the course of the scheduled waking day ($F_{6, 62} = 2.66$, $P = .023$; Table 1). The interaction "light" x "time of day" was not significant.

3.2 | Subjective variables

3.2.1 | Visual comfort

Participants rated visual comfort (ie the combined items brightness and CCT) similar for both light conditions. Also, the factor "time of day" and the interaction "light" x "time of day" were not significant.

3.2.2 | Brightness

There was neither an effect of the factor "light" nor any significant effect for the factor "time of day," but the

TABLE 1 Results of the analysis of variance for different subjective variables and cognitive performance over the time course of the study. In bold results with $P < .05$

	Analysis of variance		
	Light	**Time of day**	**Light*Time of day**
Cognitive Performance			
PVT	$F_{1,32} = 1.79$, $P = .19$	$F_{7,72} = 0.32$, $P = .94$	$F_{7,91} = 0.50$, $P = .83$
n-back	$F_{1,25} = 0.37$, $P = .55$	$\mathbf{F_{6,62} = 2.66}$, $\mathbf{P = .023}$	$F_{6,71} = 0.72$, $P = .64$
Subjective Variables			
Visual comfort	$F_{1,25} = 0.88$, $P = .36$	$F_{7,82} = 0.63$, $P = .73$	$F_{7,93} = 0.49$, $P = .84$
Brightness	$F_{1,30} = 0.87$, $P = .36$	$F_{7,83} = 1.19$, $P = .32$	$\mathbf{F_{7,92} = 3.76}$, $\mathbf{P = .001}$
CCT	$F_{1,26} = 3.57$, $P = .07$	$F_{6,71} = 0.72$, $P = .64$	$F_{7,95} = 1.59$, $P = .15$
Vigilance	$F_{1,23} = 0.08$, $P = .78$	$\mathbf{F_{7,79} = 3.81}$, $\mathbf{P = .001}$	$\mathbf{F_{7,97} = 4.76}$, $\mathbf{P = .0002}$
Concentration	$F_{1,24} = 0.06$, $P = .81$	$\mathbf{F_{7,81} = 2.29}$, $\mathbf{P = .035}$	$F_{7,97} = 1.65$, $P = .1317$
Sleepiness	$F_{1,88} = 0.62$, $P = .43$	$\mathbf{F_{40,496} = 5.69}$, $\mathbf{P < .001}$	$F_{40,504} = 1.06$, $P < .37$

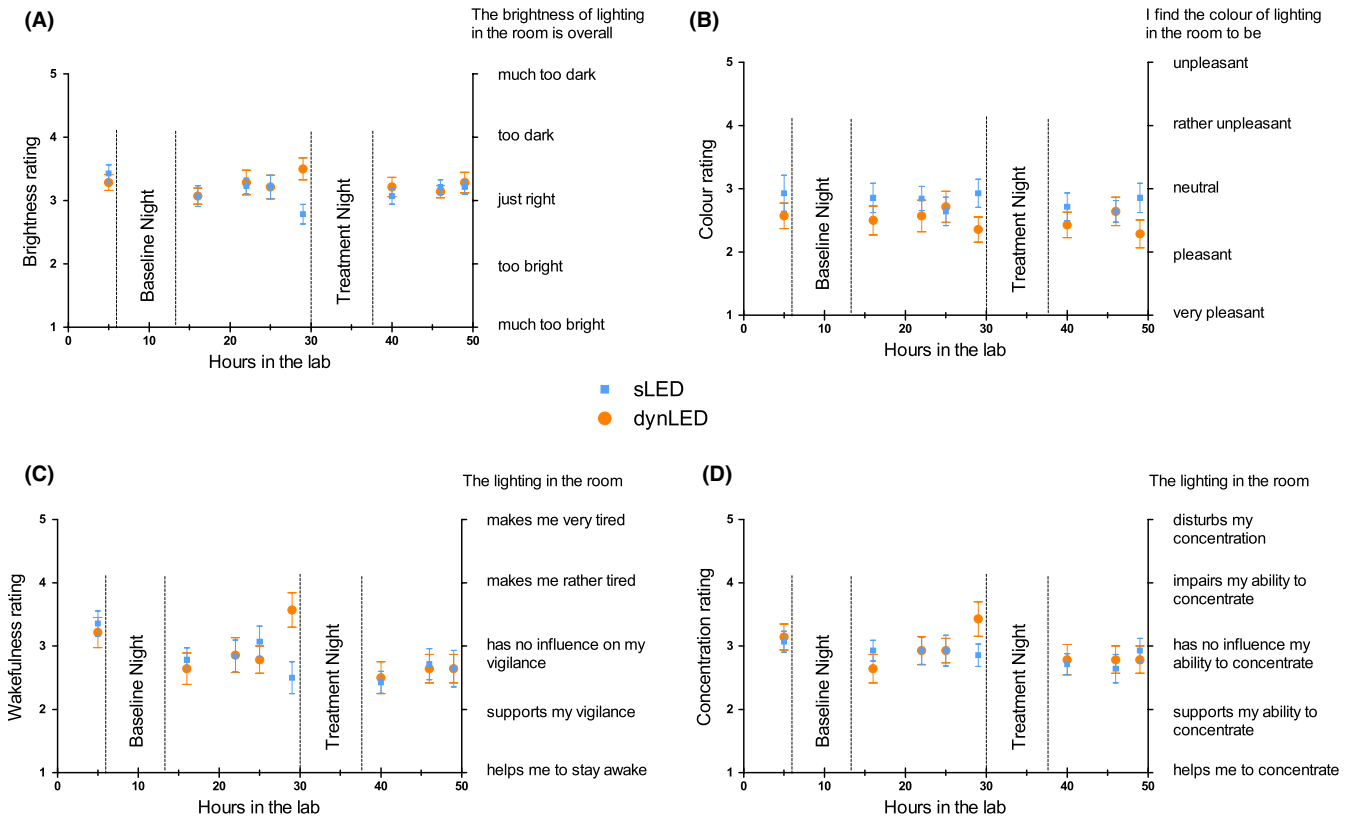


FIGURE 3 Time course of diurnal subjectively rated brightness (A), color (B), wakefulness (C), and concentration (D) for sLED and dynLED on 5-Point Likert-Scales plotted against time in hours spent in the laboratory. Depicted are the means and standard errors of the mean (n = 14). a) Brightness was rated better at 11 PM during sLED compared to dynLED (t = 4.31, $P = .004$). b) Participants trended to rate color better for dynLED (factor light: $F_{1, 26} = 3.57$, $P = .07$) than sLED. c) Participants were feeling less vigilant during the evening ($F_{7, 79} = 3.81$, $P = .001$). D) The factor "time of day" showed a significant effect with participants being less concentrated in the evening in general ($F_{7, 81} = 2.29$, $P = .035$)

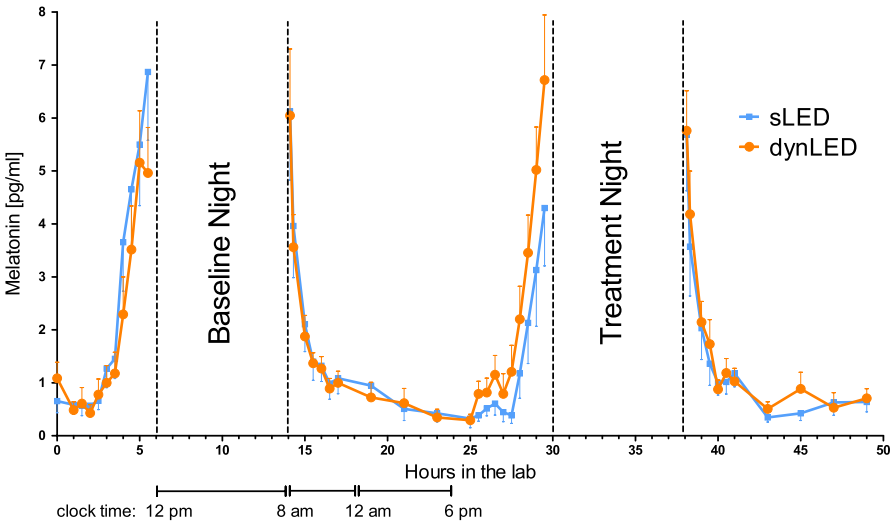


FIGURE 4 Time course of diurnal salivary melatonin profiles during the experimental conditions, sLED (blue) and dynLED (orange) in pg/mL plotted against time in hours spent in the laboratory (and clock time, ie average clock times according to the participant's habitual bedtimes). Depicted are the average melatonin levels across participants (mean values, n = 14; ±SEM) and the standard errors of the mean

interaction term "light" x "time of day" yielded significance ($F_{7, 92} = 3.76$, $P = .001$). *Post hoc* tests revealed that participants rated brightness better one hour prior to usual bedtime (ie corresponding average time of day at 11 PM) during sLED compared to dynLED (t = 4.31, $P = .004$; Figure 3A).

3.2.3 | Correlated color temperature (CCT)

Overall, participants tended to rate CCT better for dynLED (factor light: $F_{1, 26} = 3.57$, $P = .07$) than sLED. There was neither a significant main effect of "time of day" nor a significant interaction of the factors "light" and "time of day" (Figure 3B).

3.2.4 | Perception of vigilance

There was no significant main effect for the factor "light." The factor "time of day" was significant ($F_{7, 79} = 3.81$, $P = .001$) with participants feeling less vigilant during the evening. In addition, the interaction term "light" × "time of day" yielded significance ($F_{7, 97} = 4.76$, $P = .0002$). *Post hoc* comparisons indicated that volunteers felt less vigilant under dynLED compared to sLED in the evening one hour prior to usual bedtime (ie corresponding average time of day at 11 PM) (t = 3.36, $P = .035$; Figure 3C).

3.2.5 | Perception of concentration

The factor "light" did not yield significance, while the factor "time of day" showed a significant effect with participants being less concentrated in the evening ($F_{7, 81} = 2.29$, $P = .035$; Figure 3D). The interaction term "light" x "time of day" yielded no significance.

3.2.6 | Subjective sleepiness

Subjective sleepiness rated on the KSS did not significantly differ between sLED and dynLED. Sleepiness levels exhibited a typical diurnal profile with lower sleepiness during the day and increased sleepiness early in the morning and in the late evening (factor "time of day": $F_{40, 496} = 5.69$, $P < .001$). The interaction term "light" x "time of day" yielded no significance.

3.3 | Melatonin

Salivary melatonin exhibited the typical diurnal profile with falling levels in the early morning, low levels during daytime, and increasing levels in the evening (factor "time of day": $F_{41, 441} = 8.01$, $P < .0001$; Figure 4). There was no significant effect of the factor "light" on the diurnal melatonin profile ($F_{1, 85} = 0.33$, $P = .57$), while it trended to interact with the factor "time of day" ($F_{41, 460} = 1.33$, $P = .089$).

The difference of the AUC (area under the curve 4.5 hours prior to bedtime) of melatonin between baseline and treatment night was significantly larger during sLED (−5.49 pg/mL*h) compared to dynLED (+0.63 pg/mL*h) (t = 5.87, $P < .0001$; Figure 5B). In the treatment night, the AUC of melatonin during sLED (5.54 pg/mL*h) was significantly smaller than during dynLED (9.47 pg/mL*h; t = 2.82, $P = .01$; Figure 5B).

3.3.1 | Melatonin onset

The factor "light" did not show significant differences between sLED and dynLED. The factor "night" and the interaction term "light" x "night" were statistically significant ($F_{1, 12} = 12.77$, $P = .004$) and ($F_{1, 7} = 16.69$, $P = .005$), respectively. *Post hoc* tests revealed an earlier (54 min) melatonin

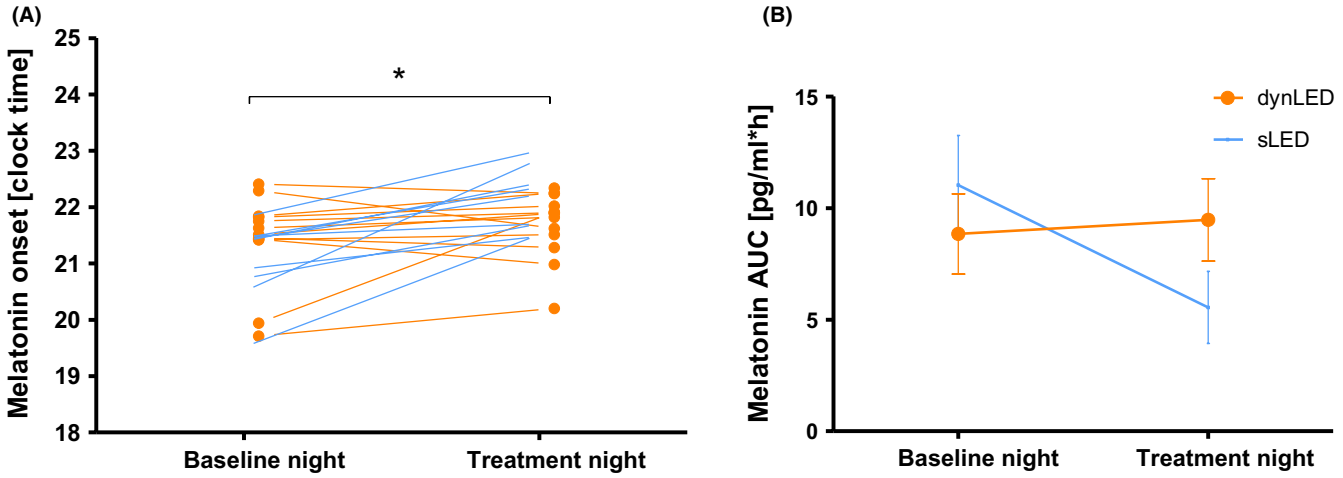


FIGURE 5 A) Melatonin onset time prior to the baseline and treatment night under sLED (blue) and dynLED condition (orange) for each participant. Melatonin onset during the baseline night was significantly earlier than during the treatment night in sLED condition (t = 4.85, $P = .008$). In the dynLED condition, such a delay between baseline and treatment night was not present. B) Melatonin AUC (area under the curve 4,5 h prior to bedtime) during the baseline and treatment night. Depicted are the means and the standard errors of the mean for the sLED (blue) and dynLED (orange) condition (n = 14)

onset during the baseline evening than during the treatment evening in sLED condition (t = 4.85, $P = .008$). Such a delay in melatonin onset from the baseline to the treatment evening was not present in the dynLED condition (9 min earlier during the baseline compared to treatment evening, nonsignificant; Figure 5A).

3.4 | Sleep

Overall, no significant main effect for the factor "light" was found for any of the sleep stages (ie N1, N2, N3, REM). The factor "night" indicated a tendency ($F_{1, 13} = 4.21$, $P = .06$) for an increase in REMS from the baseline to the treatment night. N2 and NREMS decreased significantly from baseline to treatment night ($F_{1, 13} = 5.45$, $P = .036$) and ($F_{1, 13} = 5.35$, $P = .038$), respectively. N3 was not significantly different between the nights. There was no significant interaction of the factors "light" and "night."

For sleep latency to N2, there was a significant main effect of the factor "light" ($F_{1, 13} = 5.76$, $P = .032$). The factor "night" yielded no significance ($F_{1, 13} = 1.57$, $P = .232$). There was no significant interaction of the factors "light" and "night" ($F_{1, 13} = 0.01$, $P = .929$) for sleep latency to N2. Since sleep latency to N2 was not normally distributed, we performed the nonparametric Wilcoxon signed-rank test for each night separately. During the treatment night, it took participants significantly less time to fall asleep to N2 under dynLED (13.7 min) than under sLED (17.4 min, Z = 2.13, $P = .027$; Figure 6A).

There was a significant main effect of the factor "light" for sleep latency to N1 ($F_{1, 13} = 5.01$, $P = .043$). The factor "night" yielded no significance for sleep latency to N1 ($F_{1, 13} = 1.75$, $P = .21$). There was no significant interaction of the factors "light" and "night" ($F_{1, 13} = 0.51$, $P = .487$) for sleep latency to N1. Since the distribution of sleep latency to N1 was not normal, we performed the Wilcoxon signed-rank test for each night separately. During the treatment night, participants fell significantly faster asleep to N1 under dynLED (6.8 min) than under sLED (10.9 min, Z = 2.74, $P = .003$; Figure 6A). The order of the treatment did not have a significant effect on sleep latencies.

EEG delta activity (0.75 - 4.5 Hz) during NREMS, when expressed relative to the baseline power density in the frontal EEG derivations, showed no significant effect of the factor "light." The temporal dynamics of relative frontal EEG delta activity exhibited the usual decline across the night with a superimposed ultradian NREM-REMS cycling during the baseline nights and both treatment nights, sLED and dynLED ($F_{51, 541} = 16.57$, $P < .0001$; Figure 6B). There was a significant interaction "light" x "time of day" ($F_{50, 540} = 1.56$, $P < .01$), but *post hoc* comparisons indicated no significant difference between the two light conditions.

4 | DISCUSSION

Previous research has predominantly focused on nonvisual effects of light during the night, less often during the day, and occasionally during twilight but not the combination of all three. The implementation of a dynamic lighting condition during scheduled wakefulness across a 16-h waking day resulted in less melatonin suppression, lower subjective vigilance in the evening hours, and faster sleep onset in the following sleep episode in comparison with a static lighting condition. Sleep structure, sleep quality, subjective

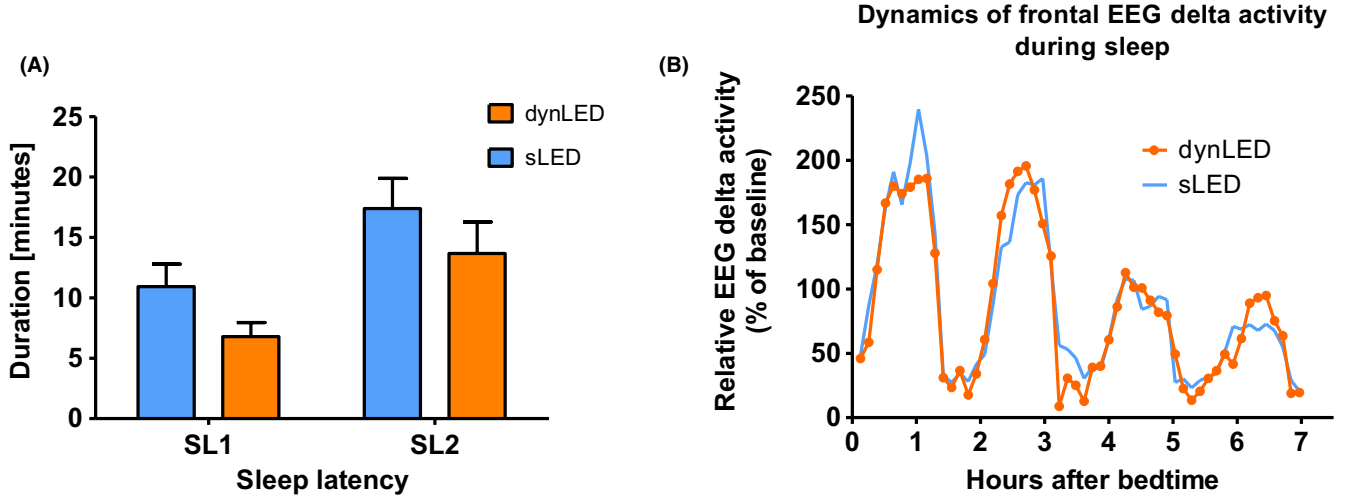


FIGURE 6 A) Sleep latencies to sleep stages N1 and N2 in minutes after lights off during the treatment night. Depicted are the means and standard errors of the mean for the sLED (blue) and dynLED condition (orange) (n = 14). B) Temporal dynamics of relative EEG delta activity (0.75 - 4.5 Hz) in NREMS during the treatment night for sLED (blue) and dynLED (orange) expressed relative to the baseline power density per participant in the frontal EEG derivations. The main effect of the factor "light" was not significant (mean values, n = 14)

sleepiness, cognitive performance, and visual comfort did not significantly differ between the two lighting conditions.

Ten percent higher melEDI (+7.1 lx) under dynLED during the day did not change cognitive performance in our volunteers, although several studies have reported light-induced alerting effects during daytime (5000 lx, melEDI 2813 lx)[36] particularly with bright light (1000 lx)[37] or of short wavelength[38] (460 nm, melEDI 64 lx). In one study with office workers working under blue-enriched fluorescent white light (17 000 K) during the day, subjective attention, positive mood, productivity, and concentration were higher than under neutral white light (4000 K).[39] Furthermore, an 18-min daytime exposure to blue appearing light (470 nm) did stimulate brain regions responsible for perception, memory, and emotions compared to green appearing light (550 nm).[40] The observed improvements are probably related to the sensitivity of ipRGCs, as magnetic resonance imaging showed that blue appearing light activates brain regions responsible for alertness and cognition even in blind individuals with intact ganglion cells.[41] Therefore, we hypothesized better daytime cognitive performance although previous studies also reported contradictory results.[42-57] It should be noted, however, that our volunteers' overall cognitive performance was rather high, making it difficult to further increase it by an environmental factor such as daytime light when the circadian pacemaker fully promotes wakefulness.[58] Subjective ratings of vigilance only differed when lighting conditions were notably different, that is at 11 PM During the late evening, participants felt rather tired, and they perceived the dynLED as being too dark. In contrast, the 1000 K increase during dynLED compared to sLED between 10 AM and 3 PM, was not large enough to be visually perceived by our volunteers, and maybe due to adaptation. A tendency ($P = .07$) for better CCT ratings during dynLED was most prominent at 11 PM, one hour prior to usual bedtime.

Our findings are consistent with previous work showing that melatonin attenuation was significantly stronger at higher CCTs and higher illuminances in the evening. We found 41.5% more attenuation of melatonin during sLED compared to dynLED, which corroborates results by Chellappa et al[59] and fits the model proposed by Prayag et al.[60] Prayag et al[60] calculated an initiation threshold for the melatonin suppression response to light at a melEDI of 1.5 lx. In the present study, melEDI during dynLED was below this level at 0.42 lx. One hour prior to bedtime, melEDI was 15-fold lower during dynLED than during sLED and 165-fold lower 10 min prior to sleep respectively. Consequently, we expected that melatonin suppression should be minimal during this time period in the dynLED condition. In the review by Prayag et al,[60] the saturation of melatonin suppression was assumed at 276 lx melEDI, and the relative melatonin suppression of 50% was calculated to be at 18.8 lx melEDI. During our sLED condition, melEDI was at 68.9 lx with 41.5% stronger melatonin attenuation compared to dynLED. The reason for less melatonin suppression at higher melanopsin weighted irradiances in the present study compared to the model by Prayag et al[60] may be due to pupil constriction (miosis). Since Prayag et al[60] analyzed a dataset from[14] in which pupils where dilated, lower light levels might have caused retinal irradiances to be higher than in our study. The present study underpins that exposure to white polychromatic LED light at 4000 K compared to light at 2700 K and lower irradiances attenuates the secretion of melatonin in the late evening. As melatonin is important for many physiological processes in the human body,[61] it should not be suppressed in the evening and night. Therefore, our results support the common recommendation

of using blue-depleted light and low illuminances in the late evening.

We hypothesized more EEG delta activity after lower CCTs (resulting in lower melEDIs) in the evening based on previous findings reporting more slow-wave activity (SWA).[62] Chellappa et al[62] investigated the effects of 2 h/40 lx compact fluorescent and incandescent light of various CCTs on sleep. After fluorescent light of 6500 K (estimated melEDI 33 lx), EEG SWA was reduced during the first sleep cycle compared to fluorescent light at 2500 K (estimated melEDI 12 lx) and incandescent light of 3000 K (estimated melEDI 20 lx). Although we compared 4.5 lx melEDI with 68.9 lx melEDI, this difference was not enough to elicit sleep alterations. With low melEDIs (low CCTs and illuminances) already starting in the late afternoon in our experiment, the buildup of homeostatic sleep pressure may have been attenuated compared to sLED condition with a constant melEDI (constant CCT and illuminance). Indeed, a recent study suggests that not only the duration of prior to wakefulness, but also the experienced illuminance during wakefulness affects homeostatic sleep regulation in humans.[63] One could therefore recommend that low melEDIs (attainable with low CCTs and low illuminances) should be administered in the late evening, but only for a very few hours before usual bedtime. Sleep latency did not change in the previous studies mentioned above (in which no dynamics were deployed). Therefore, one could assume that shorter sleep latencies as found in the present study were merely caused by the dynamics rather than the dimmer light and lower CCTs.

4.1 | Limitations

While we aimed at deploying a "naturalistic" sinusoidal illuminance change, the continuous change of CCT did not simulate a natural change during twilight but took into consideration the general recommendation of using blue-depleted light in the evening. Our study design does not allow answering, if the change in illuminance itself or the change in CCT itself would have led to the same observed effects. It does address whether a continuous change is more efficient than a square wave on/off light signal at bedtime and during wake up. We deployed this continuous change according to the recommendation not to exceed the maximum speed of CCT variation of 12K/s.[64] Despite these limitations, our data suggest that dynamic changes in illuminance and CCT mimicking "natural" dusk promote melatonin secretion and sleep initiation in healthy men.

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CONFLICTS OF INTEREST

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: KK and YS are employees of Toshiba Materials, Japan. OS is listed as an inventor on the following patents: US8646939B2—Display system having circadian effect on humans; DE102010047207B4—Projection system and method for projecting image content; US8994292B2—Adaptive lighting system; WO2006013041A1—Projection device and filter therefor; WO2016092112A1—Method for the selective adjustment of a desired brightness and/or color of a specific spatial area, and data processing device therefor. OS is a member of the Daylight Academy. OS has had the following commercial interests in the last two years (2017-18) related to lighting: Investigator-initiated research grants from Derungs, Audi, VW, Porsche, Festo, ZDF, and Toshiba; Speaker fees for invited seminars from Merck, Fraunhofer, Firalux, and Selux. CC has had the following commercial interests in the last two years (2017-2018) related to lighting: honoraria, travel, accommodation and/or meals for invited keynote lectures, conference presentations or teaching from Toshiba Materials, Velux, Firalux, Lighting Europe, Electrosuisse, Novartis, Roche, Elite, Servier, and WIR Bank. CC is a member of the Daylight Academy. MF, SV, TB, MM, and JW do not report any conflict of interest.

AUTHOR CONTRIBUTIONS

OS and CC wrote the main manuscript text. CC and YS designed the experiments. OS, MF, SV, TB, and JW carried out the study and were responsible for the study administration. OS and CC were responsible for the analysis of the data. CC served as principal investigator of the study. MM was one of the study physicians. All authors provided critical review of and revisions to the manuscript. All authors have approved the final version of this manuscript.

DATA AVAILABILITY STATMENT

The datasets analyzed during this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Schweizer C, Edwards RD, Bayer-Oglesby L, et al. Indoor time–microenvironment–activity patterns in seven regions of Europe. *J Expo Sci Environ Epidemiol*. 2007;17(2):170-181.
2. Brown TBG, Cajochen C, Czeisler C, et al. Recommendations for healthy daytime, evening, and night-time indoor light exposure. *Preprints*. 2020.

3. Ohayon M, Wickwire EM, Hirshkowitz M, et al. National Sleep Foundation's sleep quality recommendations: first report. *Sleep Health*. 2017;3(1):6-19.
4. Kozaki T, Miura N, Takahashi M, Yasukouchi A. Effect of reduced illumination on insomnia in office workers. *J Occup Health*. 2012;54(4):331-335.
5. Mason IC, Boubekri M, Figueiro MG, et al. Circadian Health and Light: A Report on the National Heart, Lung, and Blood Institute's Workshop. *J Biol Rhythms*. 2018;33(5):451-457.
6. Ohayon MM, Milesi C. Artificial outdoor nighttime lights Associate with altered sleep behavior in the american general population. *Sleep*. 2016;39(6):1311-1320.
7. Daneault V, Dumont M, Masse E, Vandewalle G, Carrier J. Light-sensitive brain pathways and aging. *J Physiol Anthropol*. 2016;35:9.
8. Benarroch EE. The melanopsin system: Phototransduction, projections, functions, and clinical implications. *Neurology*. 2011;76(16):1422-1427.
9. LeGates TA, Fernandez DC, Hattar S. Light as a central modulator of circadian rhythms, sleep and affect. *Nat Rev Neurosci*. 2014;15(7):443-454.
10. Gaggioni G, Maquet P, Schmidt C, Dijk DJ, Vandewalle G. Neuroimaging, cognition, light and circadian rhythms. *Front Syst Neurosci*. 2014;8:126.
11. Dacey DM, Liao HW, Peterson BB, et al. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. 2005;433(7027):749-754.
12. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. 2002;295(5557):1065-1070.
13. Thapan K, Arendt J, Skene DJ. An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J Physiol*. 2001;535(Pt 1):261-267.
14. Brainard GC, Hanifin JP, Greeson JM, et al. Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *J Neurosci*. 2001;21(16):6405-6412.
15. Benloucif S, Guico MJ, Reid KJ, Wolfe LF, L'Hermite-Baleriaux M, Zee PC. Stability of melatonin and temperature as circadian phase markers and their relation to sleep times in humans. *J Biol Rhythms*. 2005;20(2):178-188.
16. Cajochen C, Munch M, Kobialka S, et al. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. *J Clin Endocrinol Metab*. 2005;90(3):1311-1316.
17. Danilenko KV, Wirz-Justice A, Krauchi K, Weber JM, Terman M. The human circadian pacemaker can see by the dawn's early light. *J Biol Rhythms*. 2000;15(5):437-446.
18. Gabel V, Maire M, Reichert CF, et al. Effects of artificial dawn and morning blue light on daytime cognitive performance, well-being, cortisol and melatonin levels. *Chronobiol Int*. 2013;30(8):988-997.
19. Gabel V, Maire M, Reichert CF, et al. Dawn simulation light impacts on different cognitive domains under sleep restriction. *Behav Brain Res*. 2015;281:258-266.
20. Viola AU, Gabel V, Chellappa SL, et al. Dawn simulation light: a potential cardiac events protector. *Sleep Med*. 2015;16(4):457-461.
21. Terman M, Terman JS. Controlled trial of naturalistic dawn simulation and negative air ionization for seasonal affective disorder. *The American journal of psychiatry*. 2006;163(12):2126-2133.
22. Terman M, Schlager D, Fairhurst S, Perlman B. Dawn and dusk simulation as a therapeutic intervention. *Biol Psychiatry*. 1989;25(7):966-970.
23. de Kort Y, Smolders K. Effects of dynamic lighting on office workers: First results of a field study with monthly alternating settings. *Lighting Research & Technology*. 2010;42(3):345-360.
24. Cajochen C, Freyburger M, Basishvili T, et al. Effect of daylight LED on visual comfort, melatonin, mood, waking performance and sleep. *Lighting Research & Technology*. 2019;51(7):1044-1062.
25. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. 1989;28(2):193-213.
26. Roenneberg T, Wirz-Justice A, Merrow M. Life between clocks: Daily temporal patterns of human chronotypes. *J Biol Rhythms*. 2003;18(1):80-90.
27. Ishihara S. *Tests for color-blindness*. Tokyo: Handaya Hongo Harukich; 1917.
28. CIE. CIE System for Metrology of Optical Radiation for ipRGC-Influenced Responses to Light. CIE S 026/E:2018 Web site. http://www.cie.co.at/publications/cie-system-metrology-optical-radiation-iprgc-influenced-responses-light-0. Published 2018. Accessed2019
29. Blatter K, Graw P, Munch M, Knoblauch V, Wirz-Justice A, Cajochen C. Gender and age differences in psychomotor vigilance performance under differential sleep pressure conditions. *Behav Brain Res*. 2006;168(2):312-317.
30. Gillberg M, Kecklund G, Akerstedt T. Relations between performance and subjective ratings of sleepiness during a night awake. *Sleep*. 1994;17(3):236-241.
31. Eklund NH, Boyce PR. The development of a reliable, valid, and simple office lighting survey. *J Illuminating Engineering Society*. 1996;25(2):25-40.
32. Weber J, Schwander J, Unger I, Meier D. A direct ultrasensitive RIA for the determination of melatonin in human saliva: comparison with serum levels. *Sleep Res*. 1997;26:757.
33. Danilenko KV, Verevkin EG, Antyufeev VS, Wirz-Justice A, Cajochen C. The hockey-stick method to estimate evening dim light melatonin onset (DLMO) in humans. *Chronobiol Int*. 2014;31(3):349-355.
34. Berry RB, Brooks R, Gamaldo C, et al. AASM Scoring Manual Updates for 2017 (Version 2.4). *J Clin Sleep Med*. 2017;13(5):665-666.
35. Feinberg I, Floyd TC. Systematic trends across the night in human sleep cycles. *Psychophysiology*. 1979;16(3):283-291.
36. Ruger M, Gordijn MC, Beersma DG, de Vries B, Daan S. Time-of-day-dependent effects of bright light exposure on human psychophysiology: comparison of daytime and nighttime exposure. *Am J Physiol Regul Integr Comp Physiol*. 2006;290(5):R1413-1420.
37. Phipps-Nelson J, Redman JR, Dijk DJ, Rajaratnam SM. Daytime exposure to bright light, as compared to dim light, decreases sleepiness and improves psychomotor vigilance performance. *Sleep*. 2003;26(6):695-700.
38. Rahman SA, Flynn-Evans EE, Aeschbach D, Brainard GC, Czeisler CA, Lockley SW. Diurnal spectral sensitivity of the acute alerting effects of light. *Sleep*. 2014;37(2):271-281.
39. Viola AU, James LM, Schlangen LJ, Dijk DJ. Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. *Scand J Work Environ Health*. 2008;34(4):297-306.
40. Vandewalle G, Gais S, Schabus M, et al. Wavelength-dependent modulation of brain responses to a working memory task by daytime light exposure. *Cereb Cortex*. 2007;17(12):2788-2795.

41. Vandewalle G, Collignon O, Hull JT, et al. Blue light stimulates cognitive brain activity in visually blind individuals. *J Cogn Neurosci*. 2013;25(12):2072-2085.
42. Lok R, Smolders K, Beersma DGM, de Kort YAW. Light, Alertness, and Alerting Effects of White Light: A Literature Overview. *J Biol Rhythms*. 2018;33(6):589-601.
43. Badia P, Myers B, Boecker M, Culpepper J, Harsh JR. Bright light effects on body temperature, alertness. *EEG and behavior. Physiol Behav*. 1991;50(3):583-588.
44. Akerstedt T, Landstrom U, Bystrom M, Nordstrom B, Wibom R. Bright light as a sleepiness prophylactic: a laboratory study of subjective ratings and EEG. *Percept Mot Skills*. 2003;97(3 Pt 1):811-819.
45. Borragán G, Deliens G, Peigneux P. Chapter 45 - bright light exposure does not prevent the deterioration of alertness induced by sustained high cognitive load demands. In: Ayaz H, Dehais F (eds.), *Neuroergonomics*. Academic Press; 2018:221.
46. Huiberts LM, Smolders KC, de Kort YA. Non-image forming effects of illuminance level: Exploring parallel effects on physiological arousal and task performance. *Physiol Behav*. 2016;164(Pt A):129-139.
47. Huiberts LM, Smolders K, De Kort YAW. Seasonal and time-of-day variations in acute non-image forming effects of illuminance level on performance, physiology, and subjective well-being. *Chronobiol Int*. 2017;34(7):827-844.
48. Kaida K, Takahashi M, Haratani T, Otsuka Y, Fukasawa K, Nakata A. Indoor exposure to natural bright light prevents afternoon sleepiness. *Sleep*. 2006;29(4):462-469.
49. Leichtfried V, Mair-Raggautz M, Schaeffer V, et al. Intense illumination in the morning hours improved mood and alertness but not mental performance. *Appl Ergon*. 2015;46:54-59.
50. Lok R, Woelders T, Gordijn MCM, Hut RA, Beersma DGM. White light during daytime does not improve alertness in well-rested individuals. *J Biol Rhythms*. 2018;33(6):637-648.
51. Maierova L, Borisuit A, Scartezzini JL, Jaeggi SM, Schmidt C, Munch M. Diurnal variations of hormonal secretion, alertness and cognition in extreme chronotypes under different lighting conditions. *Sci Rep*. 2016;6:33591.
52. Sahin L, Wood BM, Plitnick B, Figueiro MG. Daytime light exposure: effects on biomarkers, measures of alertness, and performance. *Behav Brain Res*. 2014;274:176-185.
53. Smolders KC, de Kort YA, Cluitmans PJ. A higher illuminance induces alertness even during office hours: findings on subjective measures, task performance and heart rate measures. *Physiol Behav*. 2012;107(1):7-16.
54. Smolders K, Peeters ST, Vogels I, de Kort YAW. Investigation of dose-response relationships for effects of white light exposure on correlates of alertness and executive control during regular daytime working hours. *J Biol Rhythms*. 2018;33(6):649-661.
55. Te Kulve M, Schlangen L, Schellen L, Souman JL, van Marken Lichtenbelt W. Correlated colour temperature of morning light influences alertness and body temperature. *Physiol Behav*. 2018;185:1-13.
56. Vandewalle G, Balteau E, Phillips C, et al. Daytime light exposure dynamically enhances brain responses. *Curr Biol*. 2006;16(16):1616-1621.
57. Smolders KCHJ, de Kort YAW. Bright light and mental fatigue: Effects on alertness, vitality, performance and physiological arousal. *J Environmental Psychology*. 2014;39:77-91.
58. Dijk DJ, Duffy JF, Czeisler CA. Circadian and sleep/wake dependent aspects of subjective alertness and cognitive performance. *J Sleep Res*. 1992;1(2):112-117.
59. Chellappa SL, Steiner R, Blattner P, Oelhafen P, Gotz T, Cajochen C. Non-visual effects of light on melatonin, alertness and cognitive performance: can blue-enriched light keep us alert? *PLoS One*. 2011;6(1):e16429.
60. Prayag AS, Najjar RP, Gronfier C. Melatonin suppression is exquisitely sensitive to light and primarily driven by melanopsin in humans. *J Pineal Res*. 2019;66(4):e12562.
61. Majidinia MRR, Shakouri SK, Mohebbi I, et al. The multiple functions of melatonin in regenerative medicine. *Ageing Res Rev*. 2018;45:33-52.
62. Chellappa SL, Steiner R, Oelhafen P, et al. Acute exposure to evening blue-enriched light impacts on human sleep. *J Sleep Res*. 2013;22(5):573-580.
63. Cajochen C, Reichert C, Maire M, et al. Evidence that homeostatic sleep regulation depends on ambient lighting conditions during wakefulness. *Clocks Sleep*. 2019;1(4):517-531.
64. Bieske K. Dynamic Lighting - Perception of Luminous Colour Variation. Paper presented at: Proceedings of CIE 2010 Lighting Quality and Energy Efficiency 2010.

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