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Wavelength-dependent effects of evening light exposure on sleep architecture and sleep EEG power density in men

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Münch, Mirjam, Szymon Kobialka, Roland Steiner, Peter Oelhafen, Anna Wirz-Justice, and Christian Cajochen. Wavelength-dependent effects of evening light exposure on sleep architecture and sleep EEG power density in men. *Am J Physiol Regul Integr Comp Physiol* 290: R1421–R1428, 2006. First published January 26, 2006; doi:10.1152/ajpregu.00478.2005.—Light strongly influences the circadian timing system in humans via non-image-forming photoreceptors in the retinal ganglion cells. Their spectral sensitivity is highest in the short-wavelength range of the visible light spectrum as demonstrated by melatonin suppression, circadian phase shifting, acute physiological responses, and subjective alertness. We tested the impact of short wavelength light (460 nm) on sleep EEG power spectra and sleep architecture. We hypothesized that its acute action on sleep is similar in magnitude to reported effects for polychromatic light at higher intensities and stronger than longer wavelength light (550 nm). The sleep EEGs of eight young men were analyzed after 2-h evening exposure to blue (460 nm) and green (550 nm) light of equal photon densities (2.8×10^{13} photons·cm⁻²·s⁻¹) and to dark (0 lux) under constant posture conditions. The time course of EEG slow-wave activity (SWA; 0.75–4.5 Hz) across sleep cycles after blue light at 460 nm was changed such that SWA was slightly reduced in the first and significantly increased during the third sleep cycle in parietal and occipital brain regions. Moreover, blue light significantly shortened rapid eye movement (REM) sleep duration during these two sleep cycles. Thus the light effects on the dynamics of SWA and REM sleep durations were blue shifted relative to the three-cone visual photopic system probably mediated by the circadian, non-image-forming visual system. Our results can be interpreted in terms of an induction of a circadian phase delay and/or repercussions of a stronger alerting effect after blue light, persisting into the sleep episode.

monochromatic light; non-image-forming visual system; spectral analysis; sleep electroencephalogram

THE HUMAN CIRCADIAN SYSTEM is sensitive to nonvisual effects of ocular light at short wavelengths via novel photoreceptors (2, 18, 20, 22, 32). This so-called “non-image-forming” (NIF) system shows maximal response to light between 446 and 483 nm for melatonin suppression (4, 36, 39), circadian phase shifting (27, 37), and reduction in cone b-wave implicit time of the human electroretinogram (20). Subjective alertness, heart rate, and core body temperature increased significantly after blue (460 nm) but not after green light (550 nm) of equal photon density administered in the evening (8).

In contrast to the phase-shifting effects of light (12, 24, 29), less has been documented about the impact of white (polychromatic) light on human sleep architecture and spectral EEG density. Bright light in the morning shortened sleep duration (14, 15) and advanced circadian rhythms without any effects

on non-rapid eye movement (NREM) sleep homeostasis (10, 14, 15). Wakefulness accumulated faster in the night following bright light treatment (15), and sleep propensity was decreased during the first 90 min following evening light exposure, but only in the second postexposure night (10). Bright light in the evening increased sleep latency to stage 2 (5, 10, 16) and changed the time course of slow-wave activity (SWA) such that EEG delta power was lower during the first and higher during the fourth NREM-REM sleep cycle compared with the dim light condition (5). Others have reported shorter latency to REM sleep (RL) following bright light in the evening compared with dim light conditions (16) or shorter RL following morning compared with evening bright light (35). We hypothesized that changes in the sleep EEG depend on the wavelength of prior light administration. To test this hypothesis, we exposed volunteers to either monochromatic blue (460 nm) or green light (550 nm) or to a dark condition (0 lux) in the evening, 2.5 h before habitual bedtime.

METHODS

Study participants. Eight young male volunteers (age range: 20–29 yrs; mean \pm SD: 24.6 \pm 3 yrs) completed the study. All were nonsmokers free from medical, psychiatric, and sleep disorders as assessed by a physical examination and questionnaires. To exclude visual impairments and ascertain that our light application was not harmful, we carried out ophthalmologic examinations before and after the study (University Eye Clinic, Basel). For further details of the screening criteria, see Cajochen et al. (8).

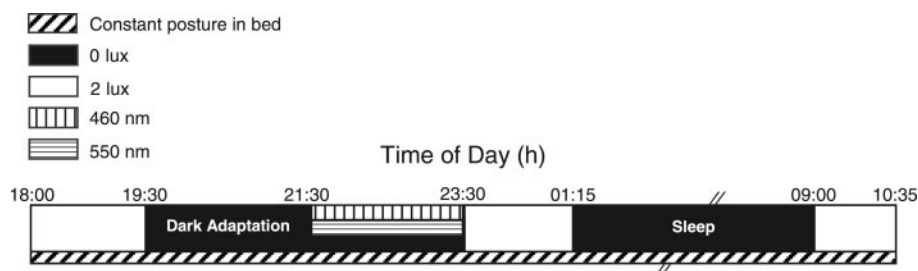
One week before the study, the volunteers were asked to abstain from excessive alcohol and caffeine consumption (i.e., at most 5 alcoholic beverages per week, and 1 cup of coffee or 1 caffeine-containing beverage per day). Furthermore, they were instructed to sleep \sim 8 h per night and to keep a regular sleep-wake schedule (with bed and wake times within \pm 30 min of self-selected target times between 10:00 PM and 2:00 AM). The outpatient segment of the study was verified using a wrist actigraph (Cambridge Neurotechnologies, Cambridge, UK), questionnaires, and self-reported sleep logs. All volunteers confirmed their compliance by written informed consent. The protocol, questionnaires, and consent form were approved by the Ethical Committee of Basel and conformed to the Declaration of Helsinki.

Study protocol and light exposure. The study consisted of three light conditions, which were carried out in weekly intervals in a balanced cross-over design with intrasubject comparisons. The volunteers were admitted to the laboratory 6.5 h before their habitual bedtime (Fig. 1). After preparation for polysomnographical sleep recordings, a constant posture protocol in bed in dim light (2 lux; polychromatic white light) was initiated. This 1.5-h episode of dim

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Fig. 1. Overview of the protocol design. The different light conditions are dark (0 lux), dim light (2 lux), blue light (460 nm), and green light (550 nm). Time of day is indicated for a subject with habitual bedtimes between 2400 and 0800. The shaded bar designates constant posture in bed (i.e. recumbent during sleep and semirecumbent during wakefulness).



light (2 lux) was followed by 2 h of dark adaptation (0 lux), and subsequently the subjects were exposed to monochromatic green (550 nm), blue (460 nm), or no light (0 lux) for 2 h. After light exposure, the subjects remained awake for another 1.75 h under dim light (2 lux). Lights off was 1.25 h after habitual bedtime, and the volunteers were allowed to sleep for 7.75 h. The rationale for shifting bedtime 1.25 h later was the requirement for monitoring the immediate aftereffects of light exposure on physiological variables (melatonin, heart rate, core body temperature, alertness) before sleep onset (see Ref. 8). During scheduled wakefulness, subjects were asked to answer numerous spoken questionnaires (visual analog scale, Karolinska sleepiness scale) and to carry out the psychomotor vigilance test. To further ensure wakefulness, a trained technician was constantly present in the adjacent room checking EEG traces on the screen display for sleep signs. Moreover, the subject's room was video controlled. Epochs of microsleep during scheduled wakefulness were also scored according to standard criteria (34) and revealed neither a significant interaction between light condition and time interval nor significant main effects of those factors ($P > 0.1$; 2-way repeated-measures ANOVA: condition \times time interval, $n = 8$; data not shown).

The monochromatic light was generated by a 300-W arc-ozone-free xenon lamp (Thermo Oriol, Spectra Physics, Stratford, CT) filtered at either 460 or 550 nm with equal photon densities for both conditions (2.8×10^{13} photons \cdot cm $^{-2}\cdot$ s $^{-1}$; interference filter, ± 10 nm half-peak bandwidth; Spectra Physics); the irradiance level was 12.1 μ W/cm 2 for blue (460 nm) and 10.05 μ W/cm 2 for green light (550 nm). The volunteers received the light via two glass fiber bundles (L.O.T. Oriol-Suisse, Romanel-sur-Morges, Switzerland) on custom-built goggles (K. Haug, Basel, Switzerland). The study protocol and the light application have been described in detail elsewhere (8).

Sleep recording and analysis. Sleep was recorded polysomnographically using the VITAPORT digital ambulatory system (Vita-port-3 digital recorder; TEMEC Instruments, Kerkrade, The Netherlands). Eight EEGs (F3, F4, C3, C4, P3, P4, O1, and O2), two electrooculograms, one submental electromyogram, and one electrocardiogram were recorded. All signals were low-pass filtered at 30 Hz (4th-order Bessel antialiasing, total 24 dB/Oct) at a time constant of 1 s before online digitization (range: 610 μ V, 12-bit analog-to-digital converter, 0.15 μ V/bit) with a sampling rate of 128 Hz (for the EEG). The raw signals were stored online on a Flash RAM card (Viking) and downloaded off-line to a personal computer hard drive.

Sleep stages were visually scored according to standard criteria (34). All EEGs were subjected to spectral analysis using a fast Fourier transformation (10% cosine 4-s window) resulting in a 0.25-Hz bin resolution. EEG artifacts were automatically detected (CASA, 2000 PhyVision, Gemert, The Netherlands). EEG power density was calculated during NREM sleep in the frequency range from 0 to 32 Hz. In this study, we report data derived from the right hemisphere (F4, C4, P4, O2) referenced against linked mastoids (A1, A2) in the frequency range between 0.5 and 20 Hz.

Thermometry. Core body temperature (CBT) was recorded continuously throughout the study, using a rectal probe with data collected every 20 s (8). For statistical analysis, CBT was averaged over 20-min intervals per subject and condition.

Statistics. The statistical packages SAS (version 6.12; SAS Institute, Cary, NC) and Statistica (version 6.1; StatSoft, Tulsa, OK) were used. Visually scored sleep stages were expressed as percentages of total sleep time or in minutes (sleep latencies, total sleep time). For the accumulation curves, sleep stages were collapsed into 15-min intervals per condition for the first 6.75 h.

Sleep EEG power density after the blue and green light exposure was expressed relative to EEG power density after the dark condition (log ratios). Relative all-night sleep EEG spectra were analyzed during the longest common NREM sleep duration (1,215 20-s epochs \approx 405 min) and retransformed as percentages of the dark condition for graphical illustration. Furthermore, SWA was accumulated for the first 6.65 h of NREM sleep (405 min), collapsed into 15-min bins, and expressed as a percentage of the accumulated value after 6.65 h during the dark condition (see Fig. 2, bottom).

NREM-REM sleep cycles were defined according to Feinberg and Floyd (17), with the exception that for the last sleep cycle, no minimum REM sleep duration was required. Thereafter, each sleep cycle was subdivided into 10 time intervals of equal length during NREM and into 4 time intervals during REM sleep and expressed as a percentage of the dark condition. One-, two-, and three-way ANOVA for repeated measures (rANOVA) with the factors "condition" (blue, green, dark), "derivation" (F4, C4, P4, O2), "cycle" (1–3), and "time interval" (15-min intervals) were used on log-transformed absolute values and on log ratios. All P values from rANOVAs were based on Huynh-Feldt's corrected degrees of freedom and adjusted for

Table 1. Visually scored sleep stages

Stage	Blue	Dark	Green	P	$F_{(2,14)}$
TST, min	407.9 \pm 11.9	390.9 \pm 11.6	391.7 \pm 13.4	NS	
SE, %	87.8 \pm 2.5	84.1 \pm 2.5	84.3 \pm 2.9	NS	
Stage 1, %	11.3 \pm 0.8	12.0 \pm 1.0	12.8 \pm 1.4	NS	
Stage 2, %	50.7 \pm 1.9	46.2 \pm 2.9	47.9 \pm 2.7	≤ 0.1	3.5
Stage 3, %	10.0 \pm 1.0	10.0 \pm 0.8	8.0 \pm 0.7	≤ 0.1	3.3
Stage 4, %	6.1 \pm 1.6	8.9 \pm 2.2	7.4 \pm 1.7	< 0.05	6.3
SWS, %	16.1 \pm 2.2	18.9 \pm 2.8	15.5 \pm 2.2	< 0.05	7.9
REM, %	21.8 \pm 1.6	22.9 \pm 1.5	23.9 \pm 1.7	NS	
NREM, %	66.8 \pm 1.4	65.1 \pm 1.2	63.4 \pm 1.3	NS	
WALO, %	12.0 \pm 2.4	14.8 \pm 2.4	14.9 \pm 2.9	NS	
Arousal, %	9.9 \pm 3.0	13.0 \pm 2.8	14.3 \pm 4.2	NS	
MT, %	1.1 \pm 0.6	1.9 \pm 0.8	1.7 \pm 0.4	NS	
SL1, min	6.9 \pm 1.5	6.5 \pm 0.9	5.3 \pm 1.1	NS	
SL2, min	8.8 \pm 1.4	9.0 \pm 1.1	7.7 \pm 1.1	NS	
RL, min	66.9 \pm 8.2	69.5 \pm 5.4	70.1 \pm 6.5	NS	

Values are means \pm SE; $n = 8$. TST, total sleep time; SE, sleep efficiency [(stages 1–4 + REM)/(time after lights off – time lights on) \times 100]; SWS, slow wave sleep (stages 3 + 4); REM, rapid eye movement sleep (% of TST); NREM, non-rapid eye movement sleep [(SWS + stage 2)/TST \times 100]; MT, movement time (after sleep onset; % of TST); WALO, wakefulness after lights off [(MT + wake)/(time after lights off – time lights on) \times 100]; arousal, wake after sleep onset (% of TST); SL1, sleep latency to stage 1; SL2, sleep latency to stage 2; RL, REM sleep latency (after sleep onset); NS, $P > 0.1$ (1-way repeated-measures ANOVA).

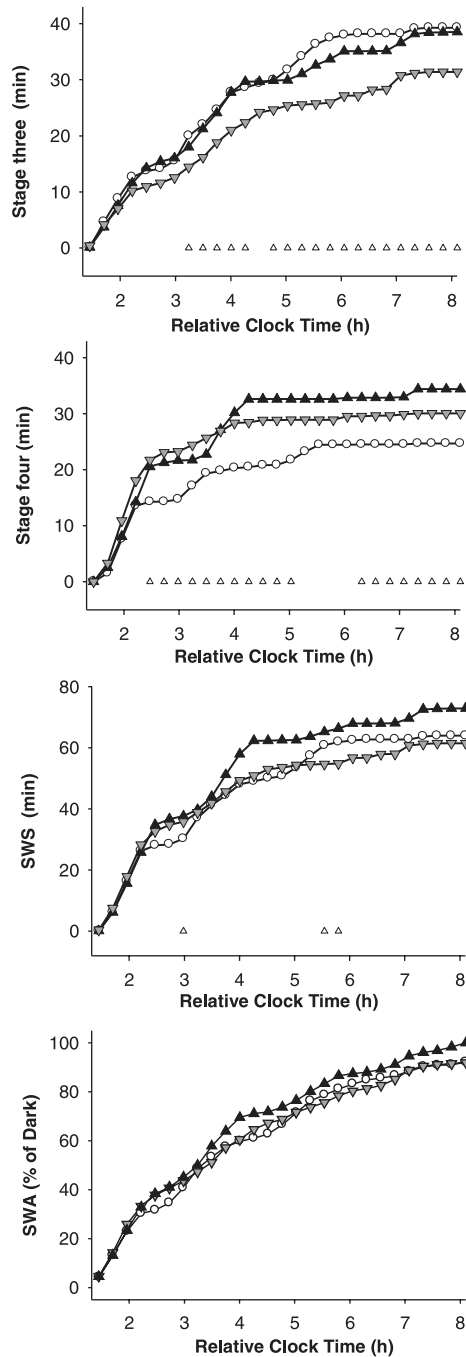


Fig. 2. Accumulation curves (collapsed into 15-min intervals for the first 6.75 h) for stage 3, stage 4, and SWS. Accumulated SWA (percentage of dark condition, collapsed into 15-min intervals) also are shown. Blue light (460 nm, \circ), green light (550 nm, grey triangle, down), and dark conditions (0 lux, black triangle) are indicated. Open triangles near the abscissa indicate intervals for which post hoc comparisons between the blue and green light conditions were significant ($n = 8$; $P < 0.05$; Duncan's multiple range test, corrected for multiple comparisons).

multiple comparisons (11). For post hoc comparisons, Duncan's multiple range test with corrections for multiple comparisons (11) was applied. All REM and NREM sleep durations per sleep cycle were analyzed using the Wilcoxon matched-pairs test, because not all of the values reached the criterion for a normal distribution.

To assess the decline of SWA (percentage of dark condition) across sleep cycles, we calculated a nonlinear regression analysis for each

subject separately. SWA was approximated using an exponential decay function: $SWA_t = SWA_\infty + SWA_0 \cdot e^{(-rt)}$, where SWA_t is averaged SWA per sleep cycle, SWA_∞ is the horizontal asymptote for time $t = \infty$, SWA_0 is the intercept on the y-axis, r is the slope of the decay, and t is the time of each NREM cycle midpoint.

RESULTS

Sleep stages. The visually scored sleep stages are summarized in Table 1. A one-way rANOVA with the factor condition yielded significance for stage 4 ($F_{2,14} = 6.3$; $P < 0.05$) and slow wave sleep (SWS; $F_{2,14} = 7.9$; $P < 0.05$) and a tendency for stages 2 and 3 ($P \leq 0.1$). Post hoc comparisons revealed significantly less stage 4 and SWS after blue light and significantly less SWS after green light compared with the dark condition ($P < 0.05$; Duncan's multiple range test). The

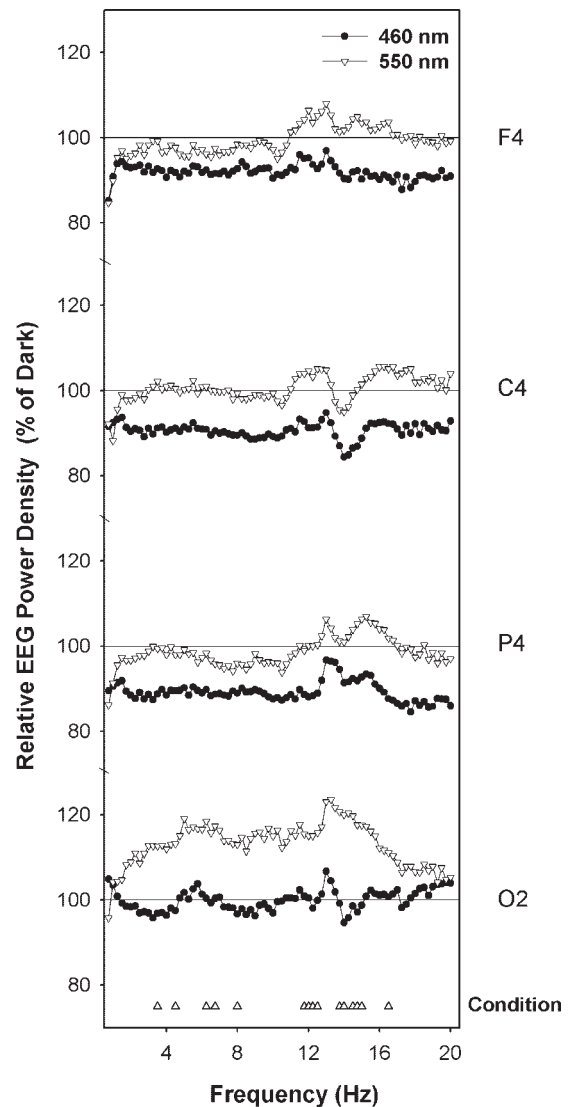


Fig. 3. Relative EEG power densities (percentage of dark mean) during non-rapid eye movement (NREM) sleep (405 min) in the frequency range between 0.5 and 20 Hz are shown for F4, C4, P4, and O2. Blue light (460 nm; black circle) and green light conditions (550 nm; white triangle) are indicated ($n = 8$). Open triangles near the abscissa indicate frequency bins for which EEG power density after green light was significantly higher than after blue light. $P < 0.05$; 1-way repeated-measures (rANOVA) performed on log ratios; P adjusted for multiple comparisons.

accumulation curves showed a significant interaction between the factors condition and time interval (1–27) for stages 3 and 4 and SWS ($F_{52,364} > 2$; $P < 0.05$). Significant post hoc comparisons between the blue and green light conditions are indicated in Fig. 2 ($P < 0.04$ for SWS and $P < 0.01$ for stages 3 and 4; Duncan's multiple range test and corrections for multiple comparisons). Hence, after green light exposure, stage 3 was significantly decreased compared with after blue light. On the other hand, stage 4 was significantly lowered after blue than after green light, and SWS was altered during three time intervals across the night, with higher values during the first and lower values during the second and third of these intervals compared with the green light condition.

Sleep spectra. A two-way rANOVA on relative EEG power density values (log ratios) with the factors condition and derivation revealed no significant interaction, and the main factor derivation yielded no significance (Fig. 3). The main factor condition yielded significance in the following frequency ranges: 3.25–3.5, 4.25–4.5, 6.0–6.25, 6.5–6.75, 7.75–

8.0, 9.0–9.25, 11.5–12.5, 13.5–14.0, 14.25–15.0, and 16.25–16.5 Hz ($P < 0.05$; corrected for multiple comparisons). Hence, relative EEG power was significantly higher for the green than the blue light condition in these frequency ranges (1-way rANOVA on log ratios, $F_{1,7}$ at least 5.6; $P < 0.05$, with the exception between 9.0 and 9.25 Hz, where $P < 0.1$). The accumulation of SWA in the course of the night (Fig. 2, bottom) was calculated during the first 6.65 h for C4 (see METHODS). There was no significant interaction with the factors time interval and condition, and the main factor condition was not significant ($P > 0.1$; 2-way rANOVA on log ratios).

Relative EEG power density per sleep cycle (percentage of respective dark cycle) for F4, C4, P4, and O2 are shown in Fig. 4. Because all subjects completed three sleep cycles, the analysis was limited to these cycles. A significant three-way interaction between the factors derivation, condition, and cycle occurred in the frequency ranges 1.25–2.75, 3.0–3.5, 3.75–4.0, 4.25–4.75, 7.75–8.25, 8.5–8.75, 11.75–12.25, 12.75–13.5, 15.75–16.0, and 17.0–17.25 Hz (rANOVA on log ratios, cor-

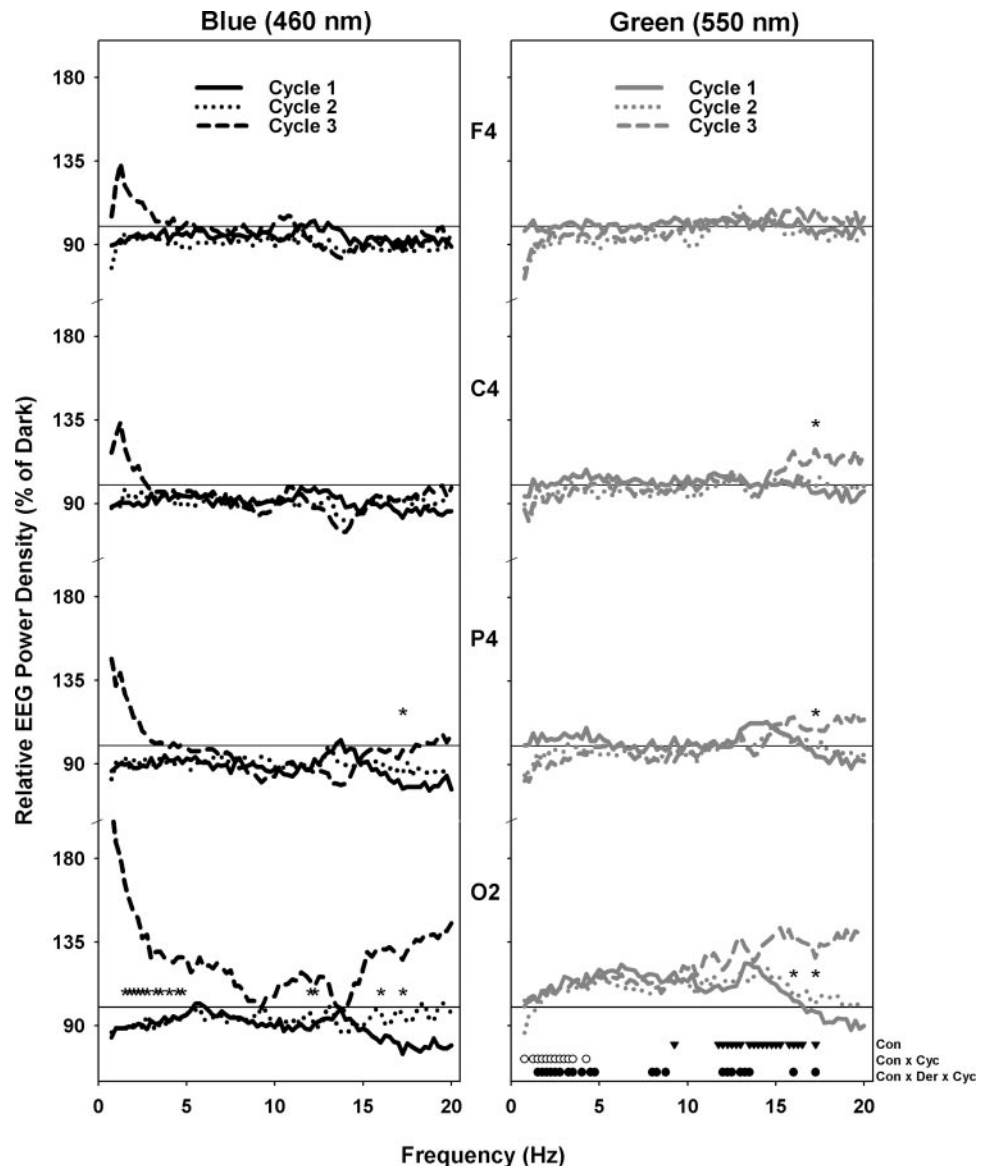


Fig. 4. Relative EEG power density per NREM sleep cycle (percentage of respective dark condition) in the frequency range between 0.5 and 20 Hz. NREM sleep cycles 1–3 for blue light (460 nm; left) and green light exposure (550 nm; right) are shown for F4, C4, P4, and O2. Filled circles at bottom right indicate frequency bins for which the 3-way interaction condition \times derivation \times cycle (Con \times Der \times Cyc) was significant; open circles indicate significant interactions for condition \times cycle, and inverted triangles represent the significant main effect of condition ($P < 0.05$, corrected for multiple comparisons). * $P < 0.05$, cycle 1 vs. cycles 2 and 3 (Duncan's multiple range test, corrected for multiple measurements).

rected for multiple comparisons, $F_{6,42}$ at least 2.6; $P < 0.05$). The interaction of factors condition \times cycle was significant in the frequency ranges 0.5–0.75, 1.0–3.5, and 4.0–4.25 Hz ($F_{2,14}$ at least 4.3; $P < 0.05$; corrected for multiple comparisons), and the main effect of condition was significant in the frequency ranges 9.0–9.25, 11.5–13.0, 13.5–15.25, and 15.5–16.5 Hz ($F_{1,7}$ at least 6.1; $P < 0.05$; adjusted to multiple measures). Post hoc comparisons revealed significant differences between the third to both the first and the second sleep cycle for SWA, and the spindle and beta frequency range (see asterisks, Fig. 4). These differences occurred mainly after blue light exposure in O2 ($P < 0.05$; Duncan's multiple range test, performed on log ratios for each derivation and condition separately and corrected for multiple comparisons).

To further analyze the time course of relative SWA across sleep cycles, we expressed SWA as a percentage of the mean dark condition and plotted values for each percentile (Fig. 5). A two-way rANOVA (condition \times cycle) performed for each derivation separately yielded significance in the parietal (P4;

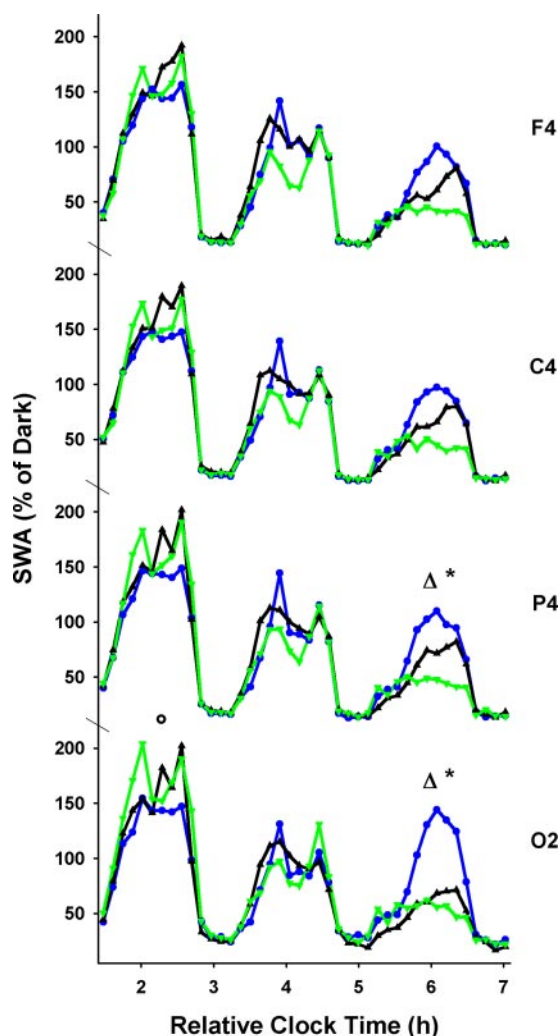


Fig. 5. Dynamics of SWA per NREM-REM sleep cycles 1–3 after sleep onset for the derivations F4, C4, P4, and O2. Values are expressed as percentages of the dark condition and are plotted against relative clock time ($n = 8$) for blue light (460 nm, blue circle), green light (550 nm, green triangle, down), and dark condition (0 lux, black triangle). * $P < 0.05$; $^{\circ}P < 0.1$, green light vs. blue light. $\Delta P < 0.05$, blue light vs. dark condition.

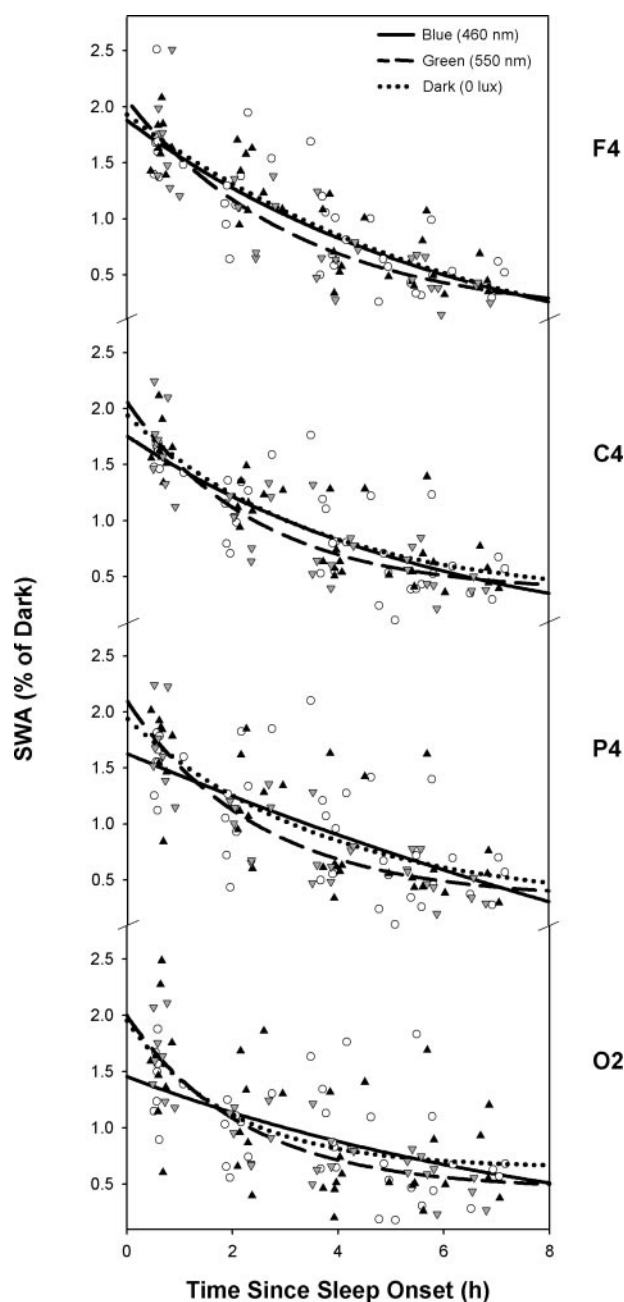


Fig. 6. Time course of mean SWA in NREM sleep per NREM-REM sleep cycle along the anteroposterior axis (F4, C4, P4, and O2). SWA was normalized to the dark condition. Solid, dashed, and dotted lines [resulting from the fitting function $SWA_t = SWA_{\infty} + SWA_0 \cdot e^{(-t/\tau)}$, see METHODS for details] indicate the regression curves for blue light (460 nm), green light (550 nm), and dark conditions (0 lux), respectively. Data points representing blue light (open circle), green light (grey triangle, down), and the dark condition (black triangle) depict mean SWA per NREM-REM cycle for each subject.

$F_{4,28} = 3.6$; $P < 0.05$) and occipital derivations (O2; $F_{4,28} = 6.1$; $P < 0.05$; performed on log ratios). The main effect of cycle was significant for F4, C4, P4, and O2 ($F_{2,14} > 28$; $P < 0.05$), whereas the factor condition was not significant ($P > 0.5$). Post hoc analysis showed a tendency for less SWA after blue light during the first sleep cycle (in O2) compared with green light ($P = 0.1$; Duncan's multiple range test) and significantly higher SWA during the third sleep cycle after blue

Table 2. Mean REM sleep durations in minutes per NREM-REM sleep cycles 1–3 for each light condition

Condition	REM Episode 1	REM Episode 2	REM Episode 3
Blue	13.5 ± 2.3*	22.9 ± 15.2	12.9 ± 4.1†‡
Dark	17.3 ± 4.5	22.1 ± 2.6	35.1 ± 9.7
Green	22.1 ± 3.3	25.7 ± 7.4	33.9 ± 9.9

Values are means ± SE; $n = 8$. * $P < 0.05$ for cycle 1, blue vs. green light condition. † $P < 0.05$ for cycle 3, blue vs. dark condition. ‡ $P < 0.1$ for cycle 3, blue vs. green light condition (Wilcoxon matched-pairs test).

light in P4 and O2 compared with green light and the dark condition ($P < 0.05$; Duncan's multiple range test).

The fitted regression curves for mean SWA per NREM-REM sleep cycle are shown in Fig. 6 for each derivation. The parameters of the decay functions revealed no significant interaction between the factors condition × derivation ($P > 0.1$; 2-way rANOVA). Only for the slopes did the main factor derivation yielded significance in O2 ($F_{3,63} = 5.1$; $P < 0.05$) compared with F4, C4, and P4 ($P < 0.05$; Duncan's multiple range test). T-tests performed between the three conditions (for each derivation separately) revealed no significance ($P > 0.1$). From visual inspection, the slope after the blue light condition in O2 appears shallower than after the green light or dark condition.

REM sleep duration (Table 2) was significantly shorter after blue light during the first sleep cycle compared with the green light condition, was significantly shorter during the third sleep cycle compared with the dark condition ($P < 0.05$), and tended to be shorter ($P = 0.06$) compared with the green light condition (Wilcoxon matched-pairs test on log-transformed data). There was no significant difference in REM sleep duration during the first sleep cycle between the green light and the dark condition ($P > 0.1$). On the other hand, NREM sleep duration was significantly shorter during the second sleep cycle after the blue light condition compared with the green light and dark conditions ($P < 0.05$; Wilcoxon matched-pairs test).

Core body temperature. The time course of CBT for each condition after the dark adaptation is shown in Fig. 7. The results of CBT before the sleep episode were reported in an

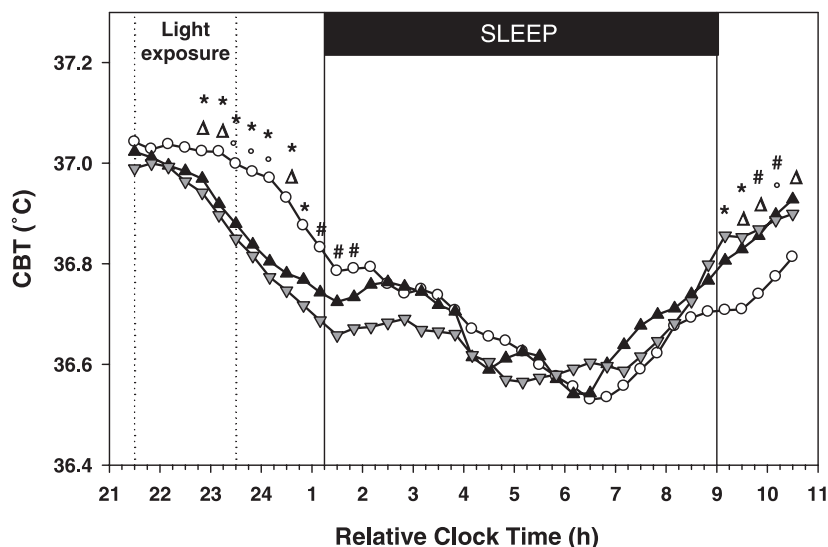
earlier publication (8). After blue light, CBT tended to be higher for the first 40 min after lights off compared with the green light condition (2-way rANOVA: condition × time interval; $F_{78,546} = 2.3$; post hoc comparisons: Duncan's multiple range test; $P < 0.1$; adjusted for multiple comparisons). The next morning, CBT was significantly lower after the blue light compared with green light during the first 40 min after lights on ($P < 0.05$; for statistics, see above) and tended to stay lower for the next 40 min. Compared with the dark condition, CBT tended to be lower for the first 40 min after lights on following blue light ($P < 0.1$) and was significantly lower for the next 20 min ($P < 0.05$) and tended to stay lower for the remainder of the study ($P < 0.1$; for statistics, see above).

DISCUSSION

Evening blue light exposure altered the time course of SWA across the postexposure sleep episode, with somewhat lower values during the first and clearly higher values during the third NREM episode, particularly in parietal and occipital EEG derivations. REM sleep duration was significantly shorter after blue light exposure than after green light during the first sleep cycle and significantly shorter than in the dark condition in the third sleep cycle.

SWA during NREM sleep is maximal early in the sleep episode, declines exponentially as sleep progresses, and is proportional to prior wake duration (3). In our study the duration of prior wakefulness was identical for all three conditions, even though the 1.25-h delay in the timing of habitual lights off slightly enhanced homeostatic sleep pressure. Therefore, the observed changes in the time course of SWA after light exposure cannot be explained by alterations in prior wakefulness. The amount of EEG-recorded microsleeps during the light exposure did not significantly differ between conditions, and when accumulated over the entire sleep episode, SWA did not statistically differ between conditions. Thus NREM sleep homeostasis does not seem to be dramatically altered by evening exposure to short wavelength light. This is also corroborated by the fact that no significant interaction between condition and derivation for EEG power density during NREM sleep was found in any of the frequency bins

Fig. 7. Time course of core body temperature (CBT) during blue light (460 nm, ○), green light (550 nm, grey triangle, down), and dark condition (0 lux, black triangle) plotted from 2130 until 1030 the next morning. * $P < 0.05$; $\Delta P < 0.1$, blue light vs. dark condition. * $P < 0.05$; # $P < 0.1$, blue light vs. green light ($n = 8$; for statistics, see text).



between 0.5 and 20 Hz. However, EEG power density was significantly reduced in some frequency bins after blue light exposure compared with the green light condition. This effect was rather unexpected and could reflect a stronger decrease of EEG power density after blue than after the green light condition, independent of EEG derivation. The slightly decreased SWA during the first and significantly increased SWA during the third sleep cycle after the blue light condition may indicate a continuation of the alerting effect found before lights off (see Ref. 8) with an intrasleep rebound of SWA during the third sleep cycle, or it may indicate the induction of a circadian phase delay. Support for the latter interpretation comes from the elevated CBT after blue light exposure, which lasted beyond the first 40 min following lights off. In addition, the shortening of the first REM sleep duration (see below), as well as the markedly lowered CBT the next morning after lights on, could favor the argument that there was an immediate phase delay after blue light exposure. However, our experiment was not designed for estimating circadian phase changes, and we did not measure any circadian phase markers 24 h after light exposure on the following day.

REM sleep undergoes a marked circadian rhythm with high values in the second half of the biological night (38). In our study, REM sleep duration did not differ between conditions across the entire night but was shortened after blue light exposure during the first and third sleep cycles. The shortening of the first REM sleep cycle may again indicate a stronger phase-delaying effect after blue than after green light. We found greater acute melatonin suppression concomitant with an increase in CBT during the evening exposure with blue light than with green light (8). This effect lasted slightly beyond light exposure and lights off (see above). Exogenous melatonin administered in the late evening to healthy young subjects has been shown to prolong the duration of the first REM episode (6, 7), along with a shorter latency to REM sleep (6). The same effect has been achieved in patients with reduced REM sleep treated with melatonin (26). However, it remains to be elucidated whether there is a causal link among melatonin suppression, increased CBT immediately before and after sleep onset, and shorter REM sleep duration during the first sleep cycle following short wavelength light exposure. The shortening of the third REM sleep cycle after blue light could be related to the definition of sleep cycles (17). According to Feinberg and Floyd (17), a REM episode must contain at least 5 min of REM sleep. However, in two subjects the third REM episode was slightly shorter than 5 min after the blue light and was counted as a REM episode because there were no REM intrusions in the following NREM sleep episode.

The hypothesis that the NIF system is involved in these wavelength-dependent effects on sleep is supported by functional connections between the melanopsin-containing retinal ganglion cells of the NIF system to the circadian pacemaker in the suprachiasmatic nuclei (2, 18, 21, 22) and to sleep-promoting neurons of the ventrolateral preoptic nucleus (VLPO) in the anterior hypothalamus (19). It has now been confirmed that melanopsin is the photopigment responsible for the NIF system (28, 30, 33). Based on the assumption that the NIF system is most sensitive to shorter wavelengths of light, its exposure would predict the strongest impact on brain areas such as the VLPO, which contains sleep-promoting neurons. Hence, the inhibition of sleep-promoting neurons would be most pro-

nounced after blue light. The (slightly) greater decrease of SWA during the first NREM cycle found after blue than after green light and the dark condition could reflect such an impact of the NIF on sleep-promoting brain areas.

It is known that polychromatic light has alerting effects in humans (1, 9, 31). Parallel analyses in the same experiment showed that 460 nm can induce such an alerting response already at these very low intensities (8). This acute effect could have lasted beyond the beginning of the sleep episode, even though any such acute alerting and/or phase-delaying effects after light exposure were not reflected in differences in sleep latencies, wakefulness after sleep onset, sleep efficiency, and total sleep time. On the other hand, sleep stages 3 and 4 were differentially affected by the two light conditions.

Differences in the time course of SWA were present only in more occipital derivations (P4, O2). If sleep is regarded not only as a global but also as a local brain phenomenon, the amount of SWA in a given brain region during sleep depends on how much it was "used" while awake (i.e., SWA increases in those brain regions that were more strongly activated during prior wakefulness) (25). Visual inputs of the image-forming system (rods, cones) reach the primary visual cortex in occipital brain regions via neuronal connections from the lateral geniculate nucleus of the thalamus (23). Opsin-containing cells of the NIF system project to the lateral geniculate nucleus (13), and therefore information from both visual systems might be processed in the visual cortex. We could speculate that after blue light exposure, the "use" of the primary visual cortex was greater than after green light or absolute darkness. However, because occipital SWA increased after blue light not at the beginning but later during the third sleep cycle, the concept of use dependency does not appear appropriate in the present study.

In conclusion, the wavelength-dependent effects of light on sleep architecture and EEG spectra were specific but rather small. They most likely resulted from an acute alerting effect continuing into sleep and/or were a consequence of an immediate phase delay induced by blue light. Our results provide further evidence that evening light exposure affects human physiology, including sleep, and is dependent not only on duration and intensity but also on its wavelength via the NIF.

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