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Bright Light Increases Alertness and Not Cortisol in Healthy Men: A Forced Desynchrony Study Under Dim and Bright Light (I)

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Abstract Light-induced improvements in alertness are more prominent during nighttime than during the day, suggesting that alerting effects of light may depend on internal clock time or wake duration. Relative contributions of both factors can be quantified using a forced desynchrony (FD) designs. FD designs have only been conducted under dim light conditions (<10lux) since light above this amount can induce non-uniform phase progression of the circadian pacemaker (also called relative coordination). This complicates the mathematical separation of circadian clock phase from homeostatic sleep pressure effects. Here we investigate alerting effects of light in a novel 4 × 18 h FD protocol (5h sleep, 13h wake) under dim (6lux) and bright light (1300lux) conditions. Hourly saliva samples (melatonin and cortisol assessment) and 2-hourly test sessions were used to assess effects of bright light on subjective and objective alertness (electroencephalography and performance). Results reveal (1) stable free-running cortisol rhythms with uniform phase progression under both light conditions, suggesting that FD designs can be conducted under bright light conditions (1300lux), (2) subjective alerting effects of light depend on elapsed time awake but not circadian clock phase, while (3) light consistently improves objective alertness independent of time awake or circadian clock phase. Reconstructing the daily time course by combining circadian clock phase and wake duration effects indicates that performance is improved during daytime, while subjective alertness remains unchanged. This suggests that high-intensity indoor lighting during the regular day might be beneficial for mental performance, even though this may not be perceived as such.

Keywords light, wake duration related variation, circadian variation, forced desynchrony, subjective alertness, performance

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The suprachiasmatic nucleus (SCN) is the pacemaker of the mammalian circadian timing system and regulates daily cycles in activity, hormonal levels, and other physiological variables (Moore and Eichler, 1972; Stephan and Zucker, 1972). The primary input of the SCN is light, and this pathway possibly plays an important role in acute alerting effects of light (Cajochen et al., 2000).

A high level of alertness is defined as a state of high sensitivity to incoming stimuli (Posner, 2008). It is known to affect many psychological and physiological functions, such as performance, caloric intake, and pain sensitivity (Alexandre et al., 2017; Curcio et al., 2001; Figueiro et al., 2015; Pardi et al., 2016). White light has been shown to significantly improve alertness during the evening and night (Cajochen et al., 2000). However, effects during daytime are less conclusive (reviewed in Lok et al., 2018a). Together, these studies implicate differences in alerting effects of light at different times of day.

Human alertness decreases with wakefulness duration, but also fluctuates independent of accumulated sleep pressure, with a cycle of approximately 24 hours (Wright et al., 2002). This indicates an influence of the SCN (Aston-Jones, 2005). The circadian drive for alertness increases during the day when homeostatic sleep pressure levels increase, resulting in relatively stable levels of alertness during the wake period (Dijk et al., 1992; Wright et al., 2002; Wu et al., 2015; Wyatt et al., 2006; Zhou et al., 2011a, 2011b). Discrepancies in alerting effects of light reported by studies investigating these effects at different times of day suggest that light effects on alertness might be modulated by circadian clock phase and/or by the amount of accumulated sleep pressure.

One approach to distinguish circadian variation from wake duration related variation is a forced desynchrony (FD) paradigm. In FD paradigms, wake and sleep periods are scheduled with a duration that deviates sufficiently from 24 h such that it falls outside the range of circadian entrainment by light. This allows the internal pacemaker to free run (i.e., following its intrinsic circadian period) throughout scheduled sleep and wakefulness (Kleitman and Kleitman, 1953). As a consequence, sleep and wake intervals are scheduled at different circadian phases, resulting in multiple combinations of homeostatic sleep drive levels and circadian phases along the FD protocol. Under certain assumptions, it is possible to mathematically disentangle wake duration related and circadian clock phase effects on parameters of interest (Dijk et al., 1992; Hiddinga et al., 1997).

Most FD protocols have only been performed in dim light (<15 lux), with the exception of Zitting et al. (2018). Light is known to phase shift the SCN, which can interfere with methods of disentangling

wake duration related variation from circadian components. This is partially caused by non-uniform phase progression of the circadian system with the sleep-wake pattern and synchronous light-dark cycle (also called relative coordination (Wever, 1977)). However, examination of the parameter space of the human circadian pacemaker indicated the possibility to run a FD design at 1300 lux without significant effects of relative coordination (Woelders et al., 2017). To investigate the contribution of both circadian and wake duration related variation to alerting effects of light, we conducted such an FD experiment in humans under both low and high light intensities. We hypothesize that alerting effects of light will depend on both wake duration related variation and circadian clock phase. Given previously reported discrepancies between objective and subjective measures of alertness, light effects will probably not be similar for subjective and objective measures (Zhou et al., 2012). To our knowledge, bright light FDs have not been previously conducted, the argument being that bright light induces a non-uniform progression of circadian phase. We show with model simulations that a 13 h wake 5 h sleep alternation yields uniform phase progression both in dim light and under 1300 lux of bright light. We hypothesized that this would also be the case in such forced desynchrony study.

MATERIALS AND METHODS

Power Calculation

Ruget al. (2005) reported an effect size of 1.9 on the Karolinska Sleepiness Scale after 1 h of bright light exposure. With alpha set to 0.05 and power to 0.8, a total of 5 participants per light condition should be included to find a statistically significant difference on the Karolinska Sleepiness Scale. To ensure sufficient power on other output parameters that are likely less sensitive, 8 participants were included (Lok et al., 2018a).

Subjects

Participants were healthy, non-sleep deprived males (n=8) between 20 and 30 years of age (average \pm SEM: 24.0 \pm 1.16). Participants provided written informed consent and received financial compensation for participation. Study protocol, screening questionnaires, and consent forms were approved by the medical ethics committee of the University Medical Center Groningen (NL54128.042) and were in agreement with the Declaration of Helsinki (2001).

An in-house developed general health questionnaire was used to assess health of the participants. As an indication of sleep timing, chronotype was assessed via the Munich Chronotype Questionnaire (MCTQ; Roenneberg et al., 2003). To determine baseline sleep quality, participants completed the Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989). Participants reported no health problems, were intermediate chronotypes (Midpoint of sleep on work-free days, sleep-corrected [MSF_{sc}] average \pm SEM: 4.88 ± 0.60), and did not report more than mild sleep problems (average \pm SEM: 4.63 ± 1.13). Exclusion criteria were (1) chronic medical conditions or the need for medication use, (2) shift work within 3 months before participation, (3) having traveled over multiple time zones within 2 months before participation, (4) smoking, (5) moderate to high levels of caffeine intake (>4 cups per day, estimated average \pm SEM was 0.75 ± 0.35 cups per day for included participants), (6) excessive alcohol use (>3 glasses per day), (7) use of recreational drugs in the last year, (8) body mass index outside the range of 18 to 27, (9) inability to complete Ishihara color blindness test (Ishihara, 1972) without errors upon arrival. Two participants reported to never drink alcohol, 2 others drank 1 to 2 glasses a month, and 4 participants reported drinking 2 to 3 glasses a week.

Protocol

Subjects were equipped with wrist worn Actigraphy (CamNtech, United Kingdom) 5 weeks before the start of the in-lab FD experiment to monitor regularity in sleep/wake cycles. On the first day of the lab protocol, participants arrived at the human isolation facility of the University of Groningen 10 h before habitual sleep onset (hson; assessed with the MCTQ). Upon arrival, individuals were equipped with EEG electrodes at the left and right frontal, central and occipital locations, electro-oculogram electrodes placed above and below both eyes, and 2 electromyography electrodes underneath the chin. Reference electrodes were placed on the left and right mastoid. Dim light melatonin onset (DLMO) was assessed through hourly saliva samples, from 7 h before hson onward. After the last saliva sample, the FD protocol started with 5 h for sleep. Participants were woken up under polychromatic white light of either dim (DL, 6 lux) or bright light intensity (BL, 1300 lux), both intensities measured vertically at the level of the eye (for more specifications of the light, see paragraph below). Subjects remained awake under these light conditions for the next 13 h (Figure 1). Two-hourly test sessions were performed during wakefulness, starting 20 min after awakening. During wakefulness, saliva samples to determine melatonin and cortisol concentrations were taken

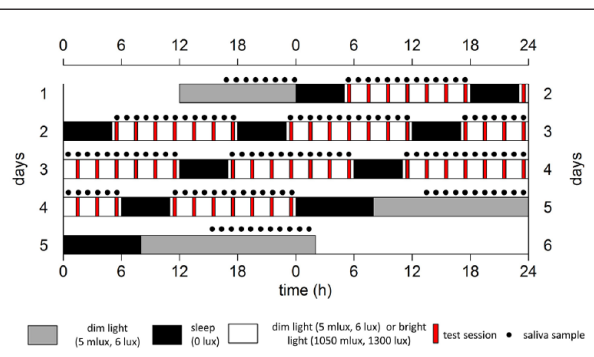


Figure 1. Schematic representation of the experiment design, for an individual with a habitual sleep onset at 00:00h, double-plotted. Gray bars indicate dim light (6 lux) conditions preceding/following the forced desynchrony protocol. Black bars represent intervals for sleep (5h, except for the last sleep attempt which is allowed to last for 8h) with lights off (0 lux), while white bars represent wakefulness in either polychromatic white dim light (6 lux, 5 melanopic lux) or bright light (1300 lux, 1050 melanopic lux) conditions. Black dots indicate saliva samples for melatonin and cortisol assessments, red bars indicate test sessions. After completion of a test session, subject received an iso-caloric snack. The protocol lasted for 72 h, therefore comprising a full beat ($3 \times 24\text{h} = 72\text{h}$, $4 \times 18\text{h} = 72\text{h}$). Color version of the figure is available online.

hourly. Iso-caloric snacks were provided immediately after completion of each test session, with caloric value based on estimated basic metabolic rate, according to the following equation: $\text{BMR} = ((10 \times \text{weight}(\text{kg})) + (6.25 \times \text{length}(\text{cm})) - (5 \times \text{age}(\text{years})) + 5)$ (Mifflin et al., 1990). After 13 h of wakefulness under dim or bright light, participants were instructed to go to bed, after which the light was switched off. The 18h FD cycle, consisting of 5h for sleep and 13h for wakefulness, was repeated 4 times, resulting in a 72h forced desynchrony protocol (4 times 18h exactly matches 3 times 24h). After completion, subjects were offered an additional sleep opportunity of 3 h. They remained in dim light in the human isolation facility. From 9 h before the following hson until 2 h after that hson, saliva samples for DLMO determination were collected. After the last sample, subjects were allowed to go home while wearing wrist Actigraphy and returned after at least 3 weeks to participate in the same protocol under opposite light conditions. The order of light conditions was counterbalanced. The experiment was conducted between February and May 2018.

Mathematical Simulations

A modified version of Kronauer's model of the human circadian pacemaker (Woelders et al., 2017) was used to model clock phase changes during a 72h ($4 \times 18\text{h}$) forced desynchrony (5 h sleep – 13 h wakefulness) under dim light (DL; 5 melanopic lux, 6 lux)

and bright light (BL; 1050 melanopic lux, 1300lux) conditions. Simulations with intrinsic circadian periods (τ) ranging from 24 to 24.6 h indicated a free running rhythm of the circadian pacemaker with uniform phase progression under both light conditions (Suppl. Fig. S1). Further simulations indicated that 5 h of sleep and 13 h of wakefulness minimized chances of non-uniform phase progression, since with this ratio, light exposure is timed in such a way that both the delay and advance zone of the phase response curve are hit equally (Suppl. Fig. S1). Simulations with the 2-process model of sleep regulation (Daan et al., 1984) allowed estimation of sleep pressure build-up under different sleep-wake durations. Accumulated sleep pressure at the end of wakefulness after 5 h sleep–13 h wakefulness was similar to a 8 h sleep–16 h wake cycle (Suppl. Fig. S2). According to the model, sleep pressure levels did not systematically increase or decrease over the course of the FD protocol (Suppl. Fig. S2).

Test Session

Test sessions started with EEG recordings (Åkerstedt and Gillberg, 1990), in which alpha and theta power density during eyes-closed (2 min) and open (6 min, while focusing on a fixation mark) were determined. Thereafter, subjects completed the Karolinska Sleepiness Scale (Åkerstedt and Gillberg, 1990), which was followed by a 5 min auditory Psychomotor Vigilance (PVT) and a 5 min auditory Go-NoGo (GNG) task, to assess sustained attention (Dinges and Powell, 1985) and executive control (Barry et al., 2014), respectively.

Light Exposure

Polychromatic white DL and BL were provided via ceiling-mounted Philips fluorescent light tubes (see Suppl. Table S1 and Fig. S3 for α -opic illuminance values and spectral composition). Each participant was exposed to the same light intensity throughout the light phase of one FD protocol.

Hormone Analysis

Saliva was collected using Sarstedt Salivettes with a cotton swab (Salivette, Sarstedt BV, Etten-Leur, the Netherlands). Melatonin concentrations were assessed by radioimmunoassay (RIA) (RK-DSM2; Bühlmann Laboratories AG, Schönenbuch, Switzerland). The limit of detection was 0.5 pg/ml with 9.1% intra-assay variation, and 18.1% inter-assay variation in lowest concentrations and 14.1% in highest concentrations. Cortisol concentrations were detected with RIA analysis (CORT-C2; Cisbio, Bioassays, Parc Marcel Boiteux,

Codolet, France), including Bio-rad Immunoassay Plus Control (Lyphochek). The limit of detection was 1 nmol/l, with 10.4% intra-assay and 9.7% inter-assay variation at low and 3.4% at high cortisol concentrations.

Tau Estimation

Tau was estimated by melatonin data, measured as DLMO (defined as crossing of the 3 pg/ml concentration, by linear interpolating of raw values before and after the cutoff point) preceding and following the FD protocol. For one individual, tau could not be assessed due to missing DLMO, resulting in $n=7$ subjects.

PVT and GNG

If a response occurred before the time calculated as the average response time of all test sessions minus two standard deviations, it was defined as an anticipation error. Errors of omission were responses occurring after the time calculated as the average of all test sessions plus two standard deviations. These definitions are the preferred method over a set cutoff point, since it yields a more accurate representation of individually varying reaction times (Gabel et al., 2019). Nevertheless, to compare results presented here to literature, a cutoff point of 500 ms was also employed. In the GNG task, errors of commission were characterized as responding to a non-response tone. Other parameters of interest were averages of the 10% fastest and 10% slowest reaction times.

EEG-Based Indices of Alertness

EEG data were collected using the Temec EEG (Vitaport, 28 channels) system. Electrode impedance was maintained below 5 k Ω at all 6 electrode locations (O3, O4, C3, C4, F3, F4). Data were analyzed using Vitascore V1.60. Artifacts were manually removed, and high-pass (0.5 Hz) and low-pass (30 Hz) digital filters were applied. Based on the Karolinska Drowsiness Test, alpha (8.0–12.0 Hz) and theta (4.0–7.9 Hz) power values during eyes-closed and eyes-open were calculated using Fast Fourier transform (FFT) with a 4 s bin width (Åkerstedt and Gillberg, 1990). Power spectra were calculated for every 30 s epoch of EEG data on every derivate.

Statistics

RStudio (version 1.0.136) was used for statistics and graphics. Wake duration related variation in performance and subjective alertness were quantified by

grouping and averaging data in 2h bins according to time since sleep offset, starting 0.5h after waking until 12h later. Wake duration related regulation of melatonin and cortisol were quantified in hourly bins. To determine circadian variation, original data were calculated as a function of circadian phase (in degrees relative to DLMO) in 30 degree bins. For optimal clarity, corresponding time of day (h) is depicted for both wake duration related and circadian variation for an individual with wake-up time of 08:00 and a DLMO of 19:00. Linear mixed models were constructed with light condition as independent variable, time since sleep offset and circadian phase as a fixed effect (categorical variable) and added interaction terms between time since sleep offset and light, and circadian phase and light condition. Subject ID and visit were included as random effects to control for between subject variation and possible order effects. Critical 2-sided significance level alpha was 0.05 for all statistical tests. To ensure sufficient sample size ($n \geq 3$) for each combination of "time since sleep offset" and "circadian clock phase," we constructed a separate linear mixed model to calculate significance of the interaction terms of these variables with light condition. Contrast analyses (comprising of a Tukey post hoc test corrected for multiple testing [Tukey correction], package "lsmeans") was conducted on all combinations of circadian time and time since sleep offset. Contrasts were constructed in 60 degree bins, with wake-dependent changes in bins of 2 h for melatonin and cortisol concentrations or 4 h for alertness parameters. Significant contrasts are depicted in 3-dimensional graphs, in which circadian variation (in 60 degree bins) is depicted on the x-axis, wake duration related variation on the y-axis, and BL scores were subtracted from DL scores, with colors indicating the direction and magnitude of the light effect. Combinations of wake duration related variation and circadian variation that contain data of fewer than 4 individuals are considered "missing data" and are depicted in grey. To estimate light effects during the time course over the regular day, sleep was predicted to start 2 h after DLMO (Burgess et al., 2003) (coinciding with circadian phase 30) and last for 5 h (until circadian phase 160), after which 13 h of wakefulness commenced. Whether light effects occurred during the projected daily time course was visually assessed.

RESULTS

Melatonin and Cortisol

Tau increased significantly (on average 21 min) after BL exposure (DL, average \pm SEM: 24.25 ± 0.09 ; BL, average \pm SEM: 24.60 ± 0.11 , $p = 2 \cdot 10^{-4}$, Suppl. Fig. S4).

Melatonin rhythms showed robust oscillations in DL, but disrupted rhythms in BL due to light-induced melatonin suppression (Figure 2a and Table 1). There was an effect of time awake on melatonin levels, with significant differences between light conditions (Figure 2b, Table 1). Significant differences between BL and DL also existed depending on circadian phases, showing dampened melatonin rhythms in BL, but significant variation in DL (Figure 2c, Table 1). These effects were even more apparent in 3D plots, with significantly higher melatonin values after DLMO (phase 0) in the DL condition, without significant differences at circadian phases when melatonin was absent (Figure 2d).

Successive cortisol cycles indicate free running of the circadian clock, without significant differences between light conditions (Figure 2e, Table 1). There were significant effects of time awake (Figure 2f, Table 1). Cortisol levels varied significantly with circadian phase, independent of light condition (Figure 2g, Table 1). There were no significant interaction effects between time and light condition (Figure 2h).

Relative Coordination

To investigate possible relative coordination, residual cortisol data were analyzed after subtraction of the calculated wake duration related variation and circadian variation. Results indicated absence of significant relative coordination, since a fitted 72h sine wave was non-significant (DL: $F_{2,359} = 0.14$, $p = 0.87$ and BL: $F_{2,359} = 0.17$, $p = 0.84$; Suppl. Fig. S5). The residual variation was respectively 1.61% and 1.83% of the variation in the raw data under DL and BL conditions, indicating that the calculated homeostatic and circadian phase fluctuations explain almost all of the variation in cortisol concentrations.

Subjective Alertness

Subjective alertness scores (Figure 3a) increased with time awake, and subjective sleepiness increased later under BL compared with DL conditions (Figure 3b). Circadian variation was established, independent of light exposure (Figure 3c, Table 1). Significant interactions were found between circadian phase, time since sleep offset, and light-induced change in subjective alertness, with increased alertness predominantly after DLMO (phase 0). The lack of statistically significant effects appears to be present during the projected daily time course (Figure 3d).

Performance: PVT

Sustained attention (defined as average reaction time on the PVT [ms], Figure 3e) was better in BL

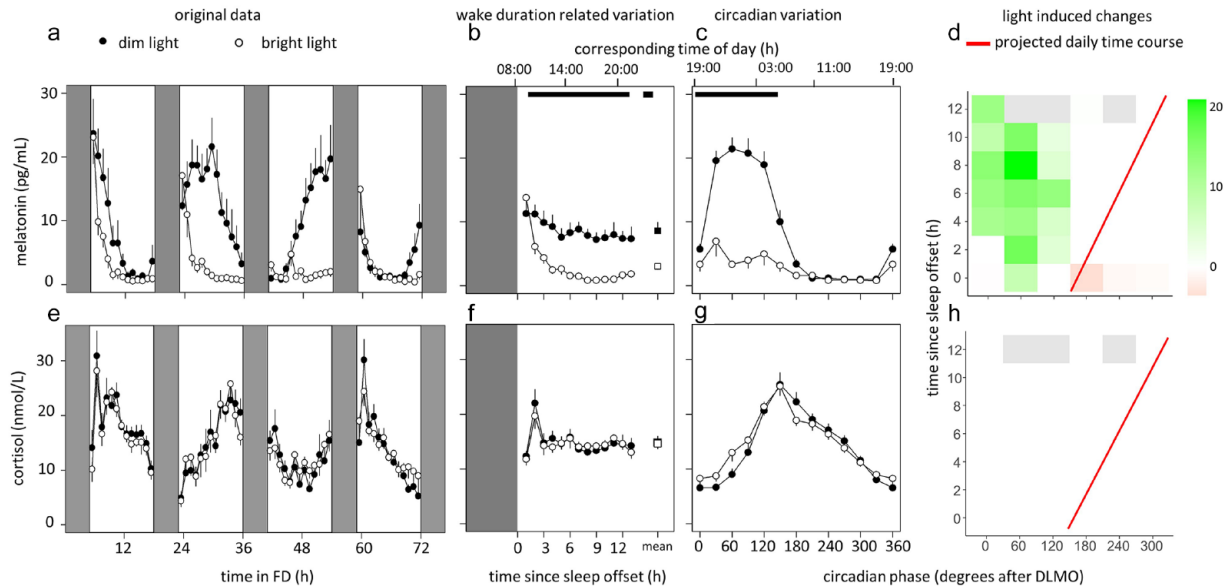


Figure 2. Data of melatonin (top panel) and cortisol (bottom panel) concentrations. Time course of melatonin (a) and cortisol (e) during the FD protocol. Data replotted as time since sleep offset (b and f) and circadian phase in degrees after DLMO (phase 0, c and g), for melatonin and cortisol respectively. Corresponding time of day (h) is depicted on the top axis. Contrast analysis describing light-induced decrease for all combinations of circadian clock phase and time since sleep offset for melatonin (d) and cortisol (h). Data represent mean \pm standard error of the mean, with 7 subjects per group. Black dots indicate data collected in dim light, white dots represent data collected in bright light and black and white squares represent averages over all data points under dim light and bright light, respectively. Red line indicates the expected time course over a regular day. Shaded areas represent scheduled sleep (at 0 lux). Significant differences between light conditions ($p < 0.05$) are indicated by horizontal black bars (b, c, f, g) or colored rectangles (d, h). Gray rectangles indicate combinations of wake duration related variation and circadian clock phase containing data of less than 4 individuals (d, h). Abbreviations: FD=forced desynchrony; DLMO=dim light melatonin onset. Color version of the figure is available online.

compared to DL, independent of time awake (Figure 3f, Table 1). There was no significant effect of circadian phase, but there were significant differences between light conditions (Figure 3g, Table 1). Significant interactions were found between circadian phase, time since sleep offset, and light-induced change in performance. Significant light-induced improvements in reaction time also occurred during the projected daily time course (Figure 3h). Detailed parameters of the PVT task can be found in Supplementary Figure S6, Table S2.

Performance: GNG

Executive performance (assessed with the GNG task, Figure 3i) was better in BL independent of time awake (Figure 3j, Table 1). There was no significant effect of circadian phase, which was affected by light exposure (Figure 3k, Table 1). There were significant interactions between circadian phase, time since sleep offset, and light-induced change in performance. Significant light-induced improvements in reaction time also occurred during the projected daily time course (Figure 3l). Detailed parameters of this task can be found in Supplementary Figure S7, Table S2. All results are summarized in Table 2.

EEG-based correlates of alertness can be found in Supplementary Figures S8-S10 and Table S3. Correlations between alertness measures can be found in Supplementary Figure S11.

DISCUSSION

Light can increase (subjective) alertness during the night, but results are less conclusive during daytime (Lok et al., 2018a). Both wake duration related variation and circadian clock phase might contribute to this discrepancy. In the current FD study, we found that light can increase performance independent of time awake, while positive effects of light on subjective alertness are only reported 6 to 9 h after waking up. High-intensity light exposure effectively postponed the increase of subjective sleepiness with progressing time awake, although light-induced improvements could not be determined (through visual assessment) during the projected daily time course. Performance was better throughout the wake interval in high-intensity light, independent of sleep pressure build-up or circadian clock phase, with significant improvements occurring during the projected daily time course.

Table 1. Summary of statistics of wake duration related variation (process S), circadian variation (process C), interaction between process S and light, C and light, and additive effects of bright light exposure.

	Wake duration related variation (process S)		Circadian variation (process C)		Interaction (process S × light)		Interaction (process C × light)		Additive effect of bright light	
Melatonin	$F_{(12, 672)}$ p	10.87, $< 2.20 \times 10^{-16}$	$F_{(5, 672)}$ p	56.41, $< 2.20 \times 10^{-16}$	$F_{(12, 672)}$ p	5.15, $< 3.12 \times 10^{-8}$	$F_{(11, 672)}$ p	24.73, $< 2.20 \times 10^{-16}$	$F_{(1, 672)}$ p	5199.52, $< 2.20 \times 10^{-16}$
Cortisol	$F_{(12, 672)}$ p	3.95, $< 7.20 \times 10^{-6}$	$F_{(5, 672)}$ p	22.18, $< 2.20 \times 10^{-16}$	$F_{(12, 672)}$ p	0.22, > 0.05	$F_{(11, 672)}$ p	0.38, > 0.05	$F_{(1, 672)}$ p	0.01, > 0.05
Subjective alertness	$F_{(6, 392)}$ p	17.87, $< 2.20 \times 10^{-16}$	$F_{(5, 392)}$ p	2.54, $< 4.40 \times 10^{-3}$	$F_{(12, 392)}$ p	1.38, > 0.05	$F_{(11, 672)}$ p	1.03, > 0.05	$F_{(1, 392)}$ p	2.27, > 0.05
PVT reaction time	$F_{(6, 392)}$ p	0.53, > 0.05	$F_{(5, 392)}$ p	0.77, > 0.05	$F_{(12, 392)}$ p	0.31, > 0.05	$F_{(11, 672)}$ p	0.32, > 0.05	$F_{(1, 392)}$ p	104.93, $< 2.20 \times 10^{-16}$
GNG reaction time	$F_{(6, 392)}$ p	0.79, > 0.05	$F_{(5, 392)}$ p	0.54, > 0.05	$F_{(12, 392)}$ p	0.36, > 0.05	$F_{(11, 672)}$ p	0.42, > 0.05	$F_{(1, 392)}$ p	88.16, $< 2.20 \times 10^{-16}$

Abbreviations: PVT=Psychomotor Vigilance; GNG=Go-NoGo. Values from linear mixed models on melatonin and cortisol concentrations, subjective alertness scores, and performance (PVT and GNG).

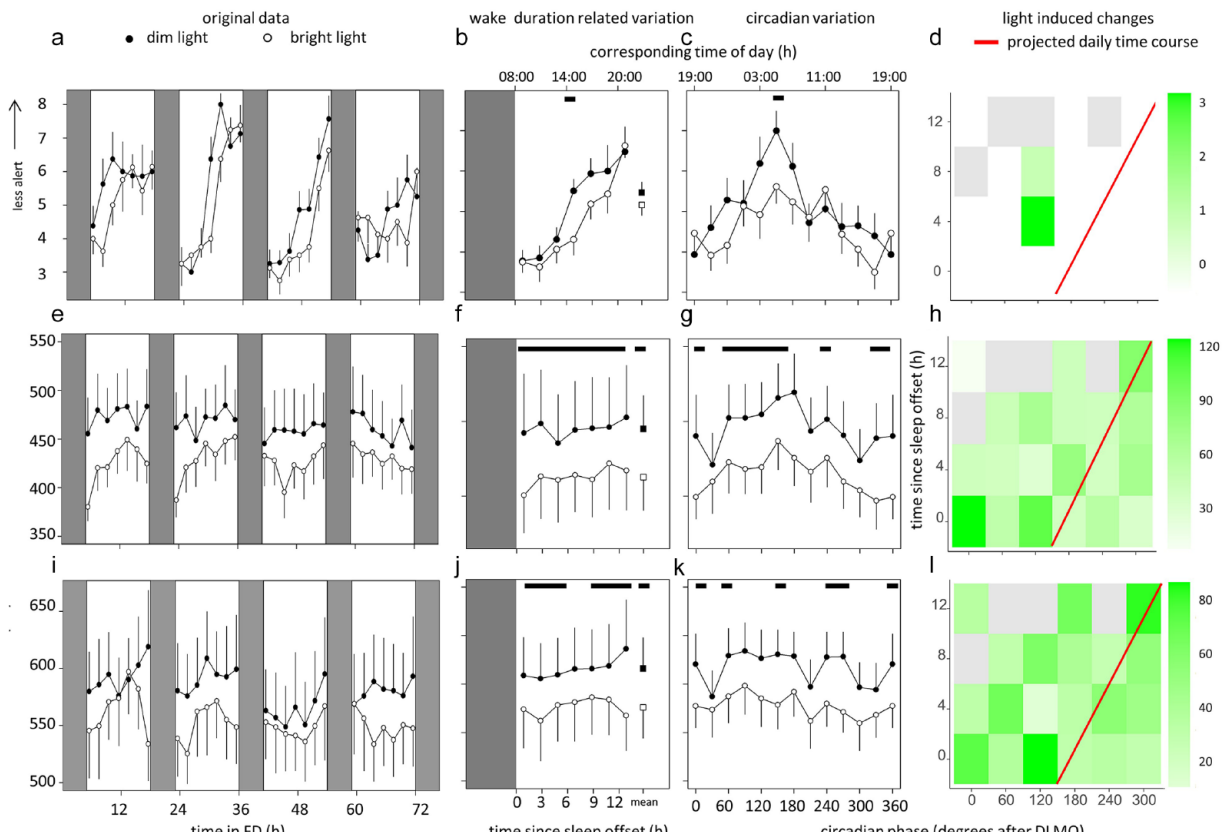


Figure 3. Data of subjective alertness (top panel), average reaction time on the PVT (middle panel) and GNG (bottom panel). Time course of subjective alertness (a), performance on the PVT (e), and GNG (i) during the FD protocol. Data replotted as time since sleep offset (b, f, and j) and circadian phase in degrees after DLMO (phase 0, c, g, and k), for subjective alertness, PVT performance, and GNG performance, respectively. Corresponding time of day (h) is depicted on the top axis. Contrast analysis describing light-induced decrease for all combinations of circadian clock phase and time since sleep offset for subjective sleepiness (d), PVT performance (h), and GNG performance (l). Data represent mean \pm standard error of the mean, with 7 subjects per group. Black dots indicate data collected in dim light, white dots represent data collected in bright light, and black and white squares represent averages over all data points under dim light and bright light, respectively. Red line indicates the expected time course over a regular day. Shaded areas represent scheduled sleep (at 0 lux). Significant differences between light conditions ($p < 0.05$) are indicated by horizontal black bars (b, c, f, g, j, k) or colored rectangles (d, h, l). Gray rectangles indicate combinations of wake duration related variation and circadian clock phase containing data of less than 4 individuals (d, h). Abbreviations: PVT=Psychomotor Vigilance; GNG=Go-NoGo; FD=forced desynchrony; DLMO=Dim light melatonin onset. Color version of the figure is available online.

Table 2. Summary of effects of wake duration related variation, internal clock time, the interaction between both processes, direction of additive effects of bright light exposure, and interaction effects between light exposure and timing of exposure during the daily time course.

	Wake Duration Related Variation (Process S)	Internal Clock Time (Process C)	Interaction (Process S × C)	Additive Effect of Bright Light	Interactive Effect During Daily Time Course
Melatonin	Y	Y	Y	–	N
Cortisol	Y	Y	Y	0	N
Subjective alertness	Y	Y	N	0	N
PVT reaction time	N	N	N	–	Y
GNG reaction time	N	N	N	–	Y

“Y” and “N” indicate significant or non-significant effects respectively, “+” indicates a significant increase, “–” represents a significant decrease, and non-significant changes are indicated by “0.”

Abbreviations: PVT = Psychomotor Vigilance; GNG = Go-NoGo.

Bright Light FD Design and Cortisol as a Phase Marker

Dim light (<10lux) has been standard procedure since the first FD experiment was performed (Kleitman and Kleitman, 1953). Apart from model calculations (Woelders et al., 2017), this is the first study we know showing that short FD protocols can also be used for disentangling effects of bright light gated by wake duration related variation and circadian processes. During FD paradigms, concentrations of the nocturnal hormone melatonin have regularly been used as output measures of SCN rhythmicity. Cortisol had to be used as circadian phase marker during this experiment, because high-intensity light suppresses melatonin production (Rollag et al., 1980). A free-running 24 h cortisol rhythm with uniform phase progression in both light conditions suggests that cortisol can be used as a reliable output measure of the circadian clock under these controlled conditions. Suggestions that light might decrease (Beck-Friis et al., 1985; Jung et al., 2010; Kostoglou-Athanassiou et al., 1998) or increase (Scheer et al., 1999) cortisol in humans are not confirmed by our data and others (Lavoie et al., 2003; McIntyre et al., 1992; Ruger et al., 2005). The absence of the cortisol awakening response in combinations of circadian clock phases and wake duration related variation employed in the current FD protocol may have contributed to the absence of light effects on cortisol. At least 54 FD studies have shown uniform phase progression under DL conditions (Archer et al., 2014; Beersma and Hiddinga, 1998; Bermudez et al., 2016; Boivin et al., 1997; Burgess and Eastman, 2008; Burke et al., 2015; Buxton et al., 2012; Cain et al., 2007; Cajochen et al., 2002; Carskadon et al., 1999; Czeisler et al., 1999; Darwent et al., 2010; Dijk and Czeisler, 1994; Dijk and Edgar, 1999; Dijk et al., 1992, 1999; Duffy et al., 2001, 2011; Ferguson et al., 2012; Gronfier et al., 2007; Hiddinga et al., 1997; Hilton et al., 2000; Hu et al., 2004; Hull et al., 2003; Ivanov et al., 2007;

Johnson et al., 1992; Kendall et al., 2001; Klerman et al., 1998; Kline et al., 2010; Koorengevel et al., 2000, 2003; Kosmadopoulos et al., 2014; Kripke et al., 2005; Lazar et al., 2015; Lee et al., 2009; Monk and Carrier, 1998; O'Donnell et al., 2009; Postnova et al., 2016; Sargent et al., 2012; Scheer et al., 2009, 2010, 2011; Shea et al., 2011; Silva et al., 2010; Waterhouse et al., 2004; Wright et al., 2002; Wright et al., 2005, 2006; Wu et al., 2015; Wyatt et al., 1999, 2004, 2006; Zhou et al., 2011a, 2011b). Since the SCN is light sensitive (Klein et al., 1991; Ralph et al., 1990), it can be expected that phase progression is less uniform in BL. If phase progression would be non-uniform in BL but uniform in DL, then cortisol concentrations would differ at sections of the cortisol curves. 3D analysis, in which cortisol concentrations between DL and BL conditions were compared point by point, indicate no significant differences between the two light conditions (Figure 2h). Hence, there is no evidence for non-uniform phase progression under BL in this FD protocol. We cannot exclude the possibility that there is non-uniform phase progression in other tissues that are under the influence of light, and that have not been measured in our FD protocol. For instance, firing rates of the SCN could be affected, with possible phase shifts as a result. Although light responsive parameters measured in this protocol do not show such reduction in circadian amplitude, we cannot exclude the possibility that changes in firing rates do occur. Nonetheless, this study presents evidence that FD designs can be conducted under high-intensity lighting.

Does Bright Light Induce Lengthening of Internal Period?

Based on the dim light melatonin onset preceding and following the FD protocol, the internal circadian period showed a 21 min increase under BL, suggesting a lengthening of the internal period (Suppl. Fig. S4).

This is in line with data observed in most mammals, including diurnal primates, lengthening showing an increase in circadian period under continuous light exposure (Klerman et al., 1996; Weber, 1967). On the other hand, diurnal vertebrates overall seem to decrease circadian period under the influence of light, as was described by Aschoff's rule and his own findings in humans (Aschoff, 1958, 1965). Earlier model simulations indicated that FD protocols with limited amount of cycles may typically overestimate circadian period at high light intensities (Woelders et al., 2017), and should therefore not be over-interpreted.

Subjective Alerting Effects of Light Depend on Homeostatic Control

Our data confirm subjective alertness increases with wakefulness duration (Dijk et al., 1992). Subjective alertness ratings were similar under both light conditions, both at the start and the end of the light phase, suggesting that the decrease in alertness is postponed by high-intensity light exposure. Subjective alertness scores after awakening were similar to natural conditions (Åkerstedt et al., 2017), suggesting that 5h for sleep, 13h wakefulness did not increase sleep pressure levels. Moreover, the relationship between alertness scores and alerting effects of light based on homeostatic sleep pressure suggest a parabolic function; a ceiling effect is present after awakening, and as a consequence alertness cannot improve beyond its limits, but with increasing sleep pressure build-up, alertness decreases, creating room for improvement by light. When sleep pressure rises further, accumulated sleep pressure overrides positive effects of bright light exposure. This hypothesis is consistent with the observations that alerting effects of light during daytime are hard to determine in well-rested individuals (Lok et al., 2018b), while significant effects have been reported in sleep deprived individuals (Phipps-Nelson et al., 2003). In addition to effects of time awake, there is also circadian variation in subjective alertness, with the largest decrease in alertness at the end of the night, as has been reported in other FD studies (Dijk et al., 1992). Most likely, this contributes to sleep maintenance when most sleep pressure has dissipated (Dijk and Czeisler, 1994; Dijk et al., 1992). Likewise, the circadian contribution to alertness at the end of the day compensates for the increased homeostatic sleep pressure at that time.

Light-Induced Performance Improvements Are Independent of Homeostatic Control

Performance was significantly improved under bright light, but did not change as a function of time

of day. This is in contrast with some (Silva et al., 2010; Wright et al., 2002) but not all literature (Lee et al., 2009; Zhou et al., 2011a), in which significant effects of time awake have been described. Similar patterns have been reported in EEG-based indices of alertness (Cajochen et al., 2002). A possible explanation for discrepancies between our and other studies might be varying lengths of FD protocols. Longer FD cycles induce higher sleep pressure levels and therefore larger declines in performance and increases in alpha-theta activity. Moreover, accumulating sleep pressure may differently affect subjective alertness, performance, and EEG (Postnova et al., 2018), as these measures do not always correlate well together (Leproult et al., 2003; Rahman et al., 2014; Van Dongen et al., 2003; Zhou et al., 2012). Our data add to the body of literature supporting this, since both performance and wake EEG reflect similar patterns, which do not coincide with subjective alertness patterns (Suppl. Fig. S11). It is noteworthy that significant improvements in performance were present after merely 20 min of high-intensity light exposure on the first day of the FD paradigm, suggesting that light effects on these parameters are relatively fast. Light exposure may counteract initial decrements in performance due to sleep inertia after awakening. The significant improvement of alertness by light in the middle of the subjective night (circadian phase 150) might be considered to be in line with findings of light-induced improvements in the early night under entrained conditions (Cajochen et al., 2005).

Limitations

First, financial and physiological limitation forced us to conduct this study in male participants only. Since body temperature, that is known to influence sleep timing (Murphy and Campbell, 1997), fluctuates more in female (Driver et al., 1996; Kolka and Stephenson, 1989) than male participants, this study only uses male participants. This clearly hinders extrapolation to other genders. Future studies should employ both genders to verify that conclusions presented in this article also account for females. Second, the relatively low number of participants could complicate statistical interpretation. However, given (1) that power calculations show that significant light effects on subjective sleepiness can already be found with merely 5 participants per light condition and (2) we report highly significant findings (Table 1), this seems unlikely. Third, the protocol duration is relatively short, encompassing merely one beat cycle. Although multiple beat cycles are desirable, a short FD design is necessary to prevent extensive light-induced tau elongations (Suppl. Fig. S4), which

could complicate comparison between the DL and BL conditions. Fourth, the chosen sleep:wakefulness ratio is 1:3, deviating from the classical 1:2 ratio. There are several reasons for this sleep-wake ratio: (1) Since uniform phase progression is essential for FD protocols, the 5:13 ratio had to be chosen, as this was the only combination that ensured equal stimulation of the phase delay and advance part of the phase response curve (Suppl. Fig. S1). (2) In addition, given this protocol encompasses merely one complete beat, the 5:13 ratio ensured sleep data at all clock phases, even when the circadian system promoted wakefulness. (3) The primary goal of this study was to compare bright versus dim light effects on human alertness, and under both protocols the 5:13 sleep to wakefulness ratio was used. Light effects are therefore still valid. Furthermore, simulations with the 2-process model of sleep show that homeostatic sleep pressure levels do not systematically increase or decrease over the 72 h of this forced desynchrony protocol (Suppl. Fig. S1).

In conclusion, this is the first study to investigate effects of light on alertness under forced desynchrony conditions, showing (1) stable free-running cortisol rhythms with uniform phase progression under both light conditions, suggesting that FD designs can be conducted under high-intensity lighting, (2) that bright light can postpone the onset of subjective alertness, depending on wake duration induced variation, and (3) that performance is improved by high-intensity light independent of wake duration related variation or circadian clock phase. Data collected here indicate that during the projected time course of a regular day, light can improve objective, but not subjective parameters of alertness. This suggests that high-intensity indoor light exposure during office hours might be beneficial for mental performance, even though this may not be perceived as such.

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AUTHOR CONTRIBUTIONS

R.L. wrote the manuscript. R.L., T.W., M.v.K., K.O., and S.G.F. were involved in data acquisition. Both R.L. and T.W. were involved in data analysis. Authors R.L., T.W., D.G.M.B., and R.A.H. contributed to the concept design, interpretation of data, and drafting of the manuscript.

CONFLICT OF INTEREST STATEMENT

The author(s) have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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NOTE

Supplementary material is available for this article online.

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