Aus dem Institut für Physiologie
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DISSERTATION

From darkness to light: Non-visual light effects can be modulated by optimizing light spectrum during nighttime and daytime

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Abstract (German)

Einführung. Neben der visuellen Wahrnehmung hat Licht auch nicht-visuelle Effekte. Diese beeinflussen den zirkadianen Rhythmus, Melatoninsuppression, Pupillenlichtreflex (PLR), Wachheit, kognitive Leistungsfähigkeit und Stimmung. Diese Dissertation basiert auf drei Studien, deren übergreifendes Ziel es war, Lichtbedingungen in Bezug auf nicht-visuelle Effekte zu verbessern: 1) Die *Nightshift Study* untersuchte gefiltertes Licht während einer Nachtschicht um Gesundheitsrisiken der nächtlichen Beleuchtung zu minimieren. 2) Die *Body Time Study* untersuchte die objektive Wachheit und kognitive Leistungsfähigkeit während einer verlängerten Wachepisode (40 Std.), um den endogenen zirkadianen Rhythmus und die Effekte von Schlafentzug zu bestimmen. 3) Die *Daytime Lighting Study* basierte auf den vorherigen Studien, indem die am besten geeigneten Messgrößen (PLR und Wach-Elektroenzephalogramm; EEG) auf ihre Tauglichkeit als Marker für nicht-visuelle Lichteffekte untersucht wurden.

Methode. In allen drei Studien kamen gesunde Versuchsteilnehmer*innen ins Labor. Sie wurden verschiedenen Lichtbedingungen oder gedimmter Beleuchtung ausgesetzt. Die Messungen beinhalteten Polysomnographie, Wach-EEG, PLR, Speichelproben, kognitive Leistungstests und subjektive Schläfrigkeitsskalen.

Ergebnisse. Die *Nightshift Study* zeigte, dass die Melatoninsekretion nicht signifikant unterdrückt oder verschoben wurde, wenn kürzerwellige Bereiche des Lichts (blau) herausgefiltert wurden. Gleichzeitig war die Wachheit erhöht im Vergleich zur Nachtschicht in gedimmtem Licht. Die *Body Time Study* zeigte in Alpha-Frequenzen des EEGs (=objektiver Wachheitsmarker) und kognitiven Leistungsfähigkeit eine Interaktion zwischen dem zirkadianen Rhythmus und homöostatischer Modulation des Schlafbedürfnisses. Die *Daytime Lighting Study* zeigte eine erhöhte objektive Wachheit am Tag, die vom Lichtspektrum und weniger von Lichtintensität abhängig war. Der PLR zeigte signifikante Unterschiede zwischen Beleuchtungsstärken und es konnte eine Dosis-Wirkungskurve für melanopische Strahlungsdichte erstellt werden.

Schlussfolgerung. Die Studien zeigen, dass Veränderungen in der spektralen Verteilung Lichtbedingungen nachts und tagsüber verbessern können. In isolierten Nachtschichten hatte gefilterte Beleuchtung positive Effekte auf die Aufrechterhaltung der Melatoninsekretion und somit auf die Gesundheit. In der zweiten Studie zeigten der homöostatische und zirkadiane Schlafdruck eine Interaktion, bei welcher gegen Ende der verlängerten Wachepisode die zirkadianen Effekte besonders deutlich hervortraten. Eine geringfügige Veränderung des Lichtspektrums tagsüber verringerte die objektive Schläfrigkeit, besonders bei einer Innenbeleuchtung mit niedriger Lichtintensität, wie sie häufig vorkommt. Der PLR zeigte sich als potentieller Marker für nicht-visuelle Lichteffekte und könnte für das Design optimaler Lichtverhältnisse in Büros, Krankenhäusern, Schulen und Wohnhäusern genutzt werden.

Abstract (English)

Introduction. Light enables vision but also has non-visual physiological effects. These include changes in phase, amplitude and period of circadian (approximately 24-h) rhythms, nighttime melatonin suppression, the pupillary light response (PLR), alertness, cognitive performance and mood. This dissertation is based on three studies, which aimed to improve lighting conditions regarding these non-visual light effects. 1) The *Nightshift Study* investigated a filtered polychromatic lighting condition during simulated nightshifts, aiming to avoid negative health effects of nighttime light exposure. 2) The *Body Time Study* investigated objective alertness and cognitive performance under constant dim light during extended wakefulness (40 h) to determine endogenous circadian rhythms and effects of sleep deprivation. 3) The *Daytime Lighting Study* expanded upon the findings of these two studies by investigating measures of interest (the PLR and wake electroencephalography; EEG) and determining whether they could be potential daytime markers of non-visual light effects.

Methods. In all three studies human participants came to the laboratory. They were exposed to different lighting conditions or constant dim light. Measurements included polysomnographic sleep recordings, wake EEG to record waking brain activity, PLR, saliva samples, cognitive performance and subjective sleepiness questionnaires.

Results. The *Nightshift Study* showed that filtering out short-wavelength (blue) light during a nightshift did not significantly suppress melatonin or induce unwanted phase shifts, while significantly increasing alertness compared to a nightshift in dim light. The *Body Time Study* showed in EEG alpha activity (=objective sleepiness marker) and cognitive performance an interaction between circadian rhythms and the homeostatic modulation of sleep need. The *Daytime Lighting Study* found significantly increased objective alertness depending on light spectrum in low illuminance daytime lighting conditions but fewer effects at higher illuminance. The PLR showed significant differences in low and higher illluminance and revealed a dose-response relationship with melanopic irradiance.

Conclusion. Changes in the spectral power distribution of light could improve lighting conditions during nighttime and daytime. In isolated nightshifts filtered lighting maintained melatonin secretion resulting in beneficial health effects. In constant dim light, the homeostatic and circadian drive for sleepiness showed an interaction, with the circadian effects becoming especially apparent after extended wakefulness. During daytime, a relatively small change in light spectrum significantly improved objective sleepiness, especially in common low illuminance indoor lighting conditions. The PLR showed that it might be an objective marker of non-visual light effects on physiology and may be used when designing optimal lighting conditions in offices, hospitals, schools and homes.

1.0 Introduction

Throughout evolution it has been an advantage to be able to anticipate the light-dark environment generated by the sun, which prompted life on earth to evolve circadian (≈ 24-h) rhythms. For thousands of years our ancestors lived under natural light conditions with full-spectrum bright light during daytime and almost complete darkness during nighttime. The control of fire was the first change to this light pattern, yet most sources of firelight provided only warm light of relatively low light intensity. People's light exposure patterns were markedly changed with the invention of artificial lighting. Gas lamps came into use during the early 1800s, light bulbs were invented in 1879, fluorescent lighting in 1900 and cold white LEDs in the 1990s (rewarded with the Nobel Prize for Physics in 2014). Due to the rise of artificial lighting people are no longer constrained in their behavior by the natural light-dark cycle of the sun. Yet, from an evolutionary perspective, this change in habitual light exposure is likely too recent for human physiology to have adapted to it. Light input to the retina of the eye is the main Zeitgeber of the biological clock in humans and an aberrant light exposure pattern that differs from the natural light exposure pattern can disrupt synchronization of processes between body functions and the outside environment (Czeisler et al., 1981). Since most processes in the body express a circadian rhythm, from the expression of immune response cells up to the behavioral level, the synchronization between the internal time and the outside 24-h day is crucial. The central biological clock, a hypothalamic brain area located in the suprachiasmatic nucleus (SCN), synchronizes circadian rhythms of the brain and body functions with the outside light-dark cycle (Czeisler et al., 1981). One of the main signals through which the SCN exerts its influence on the periphery is through stimulating the pineal gland to secrete melatonin into the bloodstream in darkness at nighttime. The increase in melatonin level in the blood during the evening prepares the periphery for nighttime [i.e., sleep; (Lavie, 1986)].

The effect that light via the retina has on the biological clock is one of the non-visual effects of light. This dissertation focuses on the non-visual effects of light, rather than the effects of light on vision. Non-visual effects of light include shifting circadian rhythms (i.e., the phase resetting and phase shifting properties of light) to synchronize the body with the outside light dark cycle, influencing the amplitude (i.e., robustness) of circadian rhythms, affecting the pupillary light response, alertness, cognition and mood. All non-visual light effects begin with light input to the retina in the eye. The human retina contains several classes of light sensitive photopigments: rhodopsin (expressed in rods), three different cone opsins (expressed in S-, M- and L-cones) and melanopsin [expressed in intrinsically photosensitive retinal ganglion cells; ipRGCs; (Hattar et al., 2002)]. While rods and cones are enabling vision, melanopsin is primarily responsible for the non-visual effects of light and therefore of particular interest in this dissertation. Melanopsin is most sensitive to short-wavelength (i.e., blue) light and has a peak sensitivity for light at wavelengths of 480 nm (Lucas et al., 2001). The ipRGCs also receive synaptic input from rods and cones, and combined they sent this light information to the SCN, as well as to the olivary pretectal nucleus (OPN) which controls the pupillary response to light. The ipRGCs have also been shown to project to many other brain nuclei including mood, learning and cognition (Fernandez et al., 2018). The precise signaling pathways for non-visual effects of light and the exact impact of different light exposure properties (e.g., spectral power distribution, intensity, timing and duration) on non-visual functions are still being elucidated.

Therefore, the effects of artificial lighting on health and well-being are still poorly understood, despite the vast worldwide implementation. There is growing evidence that light in the evening and at nighttime, especially shorter-wavelength light, negatively affects health,

as was shown by studies on shift work. Negative repercussions of aberrant light exposure patterns due to shift work have been shown in sleep disorders, depression-like symptoms, weakened immune system, increased risks of cardiovascular diseases, obesity and diabetes [for a review see: (Khan et al., 2018)].

Therefore, the goal of the first study of this dissertation (the *Nightshift Study*) was to avoid the potential negative health effects of melatonin suppression by nighttime light exposure during two simulated nightshifts. A nightshift under filtered lighting conditions (i.e., blue depleted) was compared with a nightshift in dim light (< 5 lux), regarding melatonin suppression, the phase shifting effects of light and the effects on cognitive performance. The aim was not to suppress melatonin secretion or induce phase shifts because during an isolated nightshift the internal clock should not be shifted, since it would have to immediately shift back again the next day, when the person has to perform a normal daytime shift.

The second study of this dissertation (the *Body Time Study*) used a protocol that removed nearly all external time cues to the internal clock by keeping participants in constant laboratory conditions for 40 h (i.e., constant dim light, body position in bed, temperature, no sleep and regular isocaloric meals). This study aimed to investigate the effects of circadian and homeostatic components of sleep-wake functions. Of special interest was a defined time period in the evening, when the interaction between the two components creates the so called ´Wake Maintenance Zone´ (WMZ), or 'Forbidden Zone' for sleep (Lavie, 1986). The WMZ, which occurs in the last few hours before bedtime, is characterized by a low circadian sleep pressure to counteract increasing homeostatic sleep pressure in the evening. Under normal conditions this enables a consolidated 16 h of wakefulness and 8 h of sleep. The aim was to determine the effects of the WMZ during acute sleep deprivation on EEG-derived brain activity and cognitive performance.

The third study of this dissertation (*Daytime Lighting Study*) investigated how to improve daytime lighting conditions based on information gained in the absence of light (*Body Time Study*) and the effects of light during nighttime (*Nightshift Study*). During the daytime, only few studies have investigated the impact of light on human physiology and they found inconsistent results using mainly subjective measures [for a review see (Lok et al., 2018)]. Therefore, a second aim was to find reliable objective measures that were sensitive to changes in light exposure quality and quantity during daytime. In order to be of use in designing daytime lighting conditions these objective markers had to be sensitive to different light intensities as well as changes in spectral power distribution. Such daytime markers could be used to design lighting conditions that aim to improve people's health. Two physiological parameters that were hypothesized to be potential candidates for reliable daytime markers were the pupillary light response and objective sleepiness assessed in the wake EEG.

2.0 Methods

2.1 Participants

All experiments were performed with healthy human participants. The *Nightshift Study* was performed during a practical course for medical students, who volunteered for the study after providing written informed consent. During the recruitment for the *Body Time Study* and the *Daytime Lighting Study* flyers were put up on notice boards at local universities. Volunteers who expressed interest in participating to the study filled out several screening questionnaires and underwent a medical screening. The questionnaires included two different questionnaires about chronotype (the Morning-Eveningness Questionnaire and the Munich Chronotype Questionnaire), a questionnaire about subjective sleep quality (the Pittsburgh Sleep Questionnaire Index; PSQI), and a seasonal pattern questionnaire (the Seasonal Pattern Assessment Questionnaire; SPAQ). Only moderate (neutral) chronotypes (bedtimes between 22:00 and 1:00) that had no sleep complaints (PSQI ≤ 5) and were not greatly affected by the change between seasons were included in the studies (SPAQ < 11; for references to all questionnaires see the method sections in the enclosed publications). Other inclusion criteria for participation were an age between 19 and 30 years, a BMI between 18 and 30, non-smoking, healthy, no medication use (except for oral contraceptives), no drug use or excessive alcohol intake, no shift work or travel to other time zones in the last two months. One week prior to the start of each study participants were instructed to keep regular bedtimes. Compliance was controlled by actigraphy (i.e., wearing a movement sensor on the wrist at all times to continuously record rest-activity) as well as sleep diaries.

2.2 Ethics

All three studies were approved by the ethical committee of the Charité-Universitätsmedizin in Berlin, Germany. The studies were performed according to good scientific practice and in accordance with the declaration of Helsinki 2013. Participants were informed during the screening interview and received written information on the study. They all provided written informed consent for their study participation.

2.3 Study designs

In all studies, participants were exposed to different lighting conditions (including dim light) in the laboratory under controlled conditions.

The *Nightshift Study* was performed during nighttime in groups of 4 to 5 participants (n = 24) who performed two simulated nightshifts under different lighting conditions (i.e., dim light of < 5 lux and short wavelength-depleted filtered bright light of ≈ 320 lux). The two simulated nightshifts were one week apart. Measurements included hourly cognitive performance tests and hourly saliva samples during both nightshifts to determine the lighting effects on cognition, melatonin secretion and circadian phase shifting. The participants slept in the laboratory during the nights prior to the nightshifts and during the daytime after the nightshifts.

The *Body Time Study* used a special protocol that is well established in chronobiology research, called ´constant routine´ (Mills et al., 1978). During the constant routine protocol, participants remained awake for 40 h (with one participant in the laboratory at a time; n = 12). All factors from which the body can infer external time cues (i.e., Zeitgeber) were kept constant. The participants were in dim light (< 5 lux) during the entire 40 h to minimize the effects of light, which is the strongest Zeitgeber. They remained in bed in a semi-recumbent

position to keep activity levels constant. Instead of three meals per day, participants received iso-caloric snacks of 150 kcal every hour to keep their metabolism as constant as possible throughout 40 h. Participants did not receive any clock time information during the study and a trained assistant stayed in the same room or was sitting in the next room, connected by camera and intercom. This kind of study protocol is supposed to reveal the endogenous circadian rhythms of the variables that are measured as well as the effects of sleep deprivation. Hourly measurements consisted of cognitive performance tests, saliva samples, objective sleepiness (i.e., wake EEG) and subjective sleepiness. Participants slept at the laboratory the night prior to and after the 40 h constant routine (baseline and recovery night). After the recovery night participants were exposed to 3 h of polychromatic bright light (1300 lux).

The *Daytime Lighting Study* was performed with participants who stayed in the laboratory in the morning to early afternoon (one participant per room; n = 72). All participants visited the laboratory four times and were exposed to 3 h of different lighting conditions, including a dim light control condition (< 5 lux). All visits were at least four days apart and the order of lighting conditions was randomized. A group of 24 participants was exposed to three low illuminance lighting conditions (i.e., light intensity of 100 photopic lux) which differed in spectral power distribution (i.e., low or high melanopsin activation: low-mel, high-mel and highest-mel). Two other groups of 24 participants each were exposed to three different lighting conditions at higher illuminances (i.e., 200, 600 and 1200 photopic lux) and at one out of two spectral power distributions (i.e., low-mel or high-mel). The effects of the different daytime lighting conditions on objective alertness (waking EEG) and the pupillary light response were measured.

2.4 Light exposure

Light exposure conditions can be described in several ways, however, recently recommendations were made on how to report light exposure data in order to allow comparisons between different studies (Spitschan et al., 2019). Specifications of lighting conditions included in the three studies of this dissertation are intensity (i.e., illuminance; lux = lumen/m^2), irradiance (W/m^2), luminance (cd/m^2), color temperature (Kelvin) and spectral composition (i.e., spectral power distribution across wavelengths in the visible range between 380 and 700 nm). The light intensity is usually expressed in photopic lux, which is a metric weighted for human color vision. More recently, weighted illuminance has been determined for each photoreceptor [i.e., alpha-opic light intensities: rhodopic, melanopic, S-cone-opic, M-cone-opic and L-cone-opic lux; (Lucas et al., 2014)]. Likewise, irradiance can also be expressed as photopic irradiance or photopigment specific alpha-opic irradiance (see also Figure 3 and Table 1 of the *Daytime Lighting Study*). The description of lighting conditions can be further complemented by giving the timing, duration, whether the light exposure is constant or dynamic, and whether it is measured vertically at eye level or at a horizontal plane. The lighting conditions in the studies of this dissertation were all measured vertically at eye level in the direction of gaze.

2.5 Saliva samples

In the *Nightshift Study* and *Body Time Study*, melatonin concentrations were determined from hourly taken saliva samples. Since melatonin is only expressed during nighttime and only in darkness (< 5 lux) it was not sampled during the *Daytime Lighting Study*, which was performed in the late morning to early afternoon. The saliva samples of the *Nightshift Study* and the *Body Time Study* were sent to an external laboratory where they were assessed via

radioimmunoassay (IBL International GmbH, Hamburg, Germany). Since melatonin is the main signal from the SCN to the periphery to convey time of day and synchronize circadian rhythms, it can be used to determine the phase of the endogenous clock (i.e., internal time). A standard method to assess internal time is by determining the onset of melatonin secretion, called the dim light melatonin onset (DLMO). This is the time when the melatonin concentrations (in saliva or blood) either reach a certain fixed threshold (10 pg/mL in the Nightshift Study), or a threshold of two standard deviations greater than three preceding low daytime concentrations (used in the *Body Time Study*). Comparing the timing of the DLMOs between two or more subsequent evenings is used as a proxy for whether there has been a circadian phase shift of the endogenous clock.

2.6 Pupillometry

We measured the pupillary light response during the *Daytime Lighting Study* in non-dilated pupils. Each pupil recording lasted 35 s during which participants were exposed to two different short-wavelength (i.e., blue) light pulses of 1-s duration. A handheld pupillometer was placed at the level of the right eye. The participants were asked to cover their left eye with their left hand, so that they received no light apart from the light pulses of the pupillometer. The recordings started with 3 s complete darkness. Then participants were exposed to the first 1-s light pulse followed by 15 s in darkness. This was followed by a second 1-s light pulse and again 15 s darkness. In response to each light pulse the maximum pupil constriction was determined, also called the maximum contraction amplitude (maximum CA). This is the immediate response to light stimuli, which has been shown to be mainly under the control of rods and cones. The pupil diameter 6 s after the end of the light pulse (during the redilation phase in darkness) was also determined, which is called the post-illumination pupil response (PIPR; see also Figure 2 of the *Daytime Lighting Study*). The PIPR has been shown to be mainly affected by activity of ipRGC (Gamlin et al., 2007).

2.7 Polysomnography & Wake EEG

2.7.1 Polysomnography

During the *Nightshift Study* participants slept in the laboratory before and after the nightshift. And during the *Body Time Study* participants slept in the laboratory before and after the 40 h constant routine. Their sleep was recorded by polysomnography which included electrodes to record electrical brain activity (electroencephalogram; EEG), eye movements (electrooculography; EOG), muscle tone (electromyography; EMG), heart rate (electrocardiogram; ECG), and leg electrodes for leg muscle movements. We recorded the polysomnography to assess the effects on sleep of the two nightshifts performed under different lighting conditions (*Nightshift Study*), or to assess the effects of sleep deprivation (*Body Time Study*) on subsequent sleep. During the *Body Time Study* all participants underwent an additional adaptation night prior to the study day to ensure that only healthy sleepers were included in the study (no periodic leg movements, < 10/h; no apnoea/hypopnea, < 15/h).

2.7.2 Wake EEG

We measured the wake EEG in the *Body Time Study* and the *Daytime Lighting Study*. Every hour participants performed a Karolinska Drowsiness Test (KDT) during which they had to refrain from moving and had to blink as rarely as possible in order to reduce movement and blink artifacts in the EEG. During the *Body Time Study* the KDTs were

performed for 3 min with eyes open. Six EEG derivations (i.e., electrode positions on the head) were measured, with three electrodes on the left and three on the right side of the head (i.e., two frontal, central and occipital derivations). During the *Daytime Lighting Study* two parietal derivations were added and the KDTs were performed for 5 min with open eyes, followed by 5 min with closed eyes. The brain activity in the frequency range from 0.5 to 25 Hz was of particular interest. This range can be subdivided into EEG delta (0.5 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 13 Hz), sigma (13 – 15 Hz), and beta (15 – 25 Hz) activity. Higher power densities of especially the low frequency ranges (i.e., EEG delta and theta activity) have been shown to increase with time awake, indicating a higher sleepiness (Finelli et al., 2000). The ratio of EEG alpha activity in closed eyes and open eyes, defined as the Alpha Attenuation Test index (AAT index), has been previously validated as a marker for sleepiness (Stampi et al., 1995).

2.8 Cognitive tests and questionnaires

In the *Nightshift Study* the psychomotor vigilance test (PVT) was performed hourly. In the *Body Time Study* participants performed a battery of cognitive tests on a laptop every hour (see the method section of the publication for references to the cognitive tests). The cognitive performance tests included tests for attention (PVT), response inhibition (Go/No-Go), working memory (N-back tests and addition task), memory recall (word-pair memory test), emotional processing (negative affect test) and executive functioning (abstract reasoning).

Two questionnaires were used to score subjective sleepiness. During the *Nightshift Study* and the *Daytime Lighting Study* the visual analogue scale (VAS) was performed. Participants had to indicate how sleepy they were on a line of 10 cm, from sleepy to alert. During the *Body Time Study* the Karolinska Sleepiness Scale (KSS) was performed, with participants indicating every hour how sleepy they were on a scale from 1 to 9.

2.9 Statistics

Statistics were performed with IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp., Armonk, N.Y., USA). For data that showed a normal distribution linear mixed models were performed. Non-parametric tests (e.g., Wilcoxon signed-rank test) were used when data did not show a normal distribution. Curve fitting was performed using SigmaPlot© Version 11.0, Systat Software Inc.©, San Jose, California/USA. For more details, see the statistics paragraph of each enclosed publication.

3.0 Main results

3.1 Study 1: The Nightshift Study

Light exposure during the night is known to suppress melatonin, but filtering out the short-wavelength (i.e., blue) light in the *Nightshift Study* prevented this suppression (see Table 1 below; and see Figure 1 of the publication). Compared to the simulated nightshift in the dim light control condition there was no significant melatonin suppression under filtered light. When comparing the DLMOs between the evenings prior to the nightshift and after the nightshift, the *Nightshift Study* showed that the filtered lighting condition did not induce a phase shift of the melatonin rhythm (see Table 1 below; and see Figure 2 of the publication). In addition, the filtered lighting significantly improved cognitive performance in the second half of the nightshift (see Table 1 below; and see Figure 3 of the publication).

	Nightshift in Dim Light	**Nightshift in Filtered Light**	**F and p-values**
Melatonin Suppression	AUC of 224.98 ± 97.93	AUC of 207.16 ± 98.17	$F_{1,22} = 2.630$, $p = 0.119$
Phase Shift	Phase delay of 6 ± 29 min	Phase advance of 15 ± 30 min	ns $p = 0.100$
Cognitive Performance	Reaction Time of 379 ± 29 ms	Reaction Time of 364 ± 33 ms	$F_{1,39} = 3.600$, $p = 0.038$

Table 1. Main results of the Nightshift Study. This table shows the three main results of the Nightshift Study: no significant melatonin suppression (n = 23), no significant phase shift (n = 11), and significantly improved cognitive performance (during the second half of the night; n = 24) in the filtered lighting condition compared to the dim light control condition. Column two and three show means with standard deviations. Column four shows the F and p-values. A Wilcoxon signed-rank test was performed on the not normally distributed phase shift data. AUC = Area Under the Curve. ns = not significant.

3.2 Study 2: The Body Time Study

This study revealed in the absence of external Zeitgeber (i.e., time cues) the interaction between the endogenous circadian rhythm and the homeostatic change of cognition and EEG brain activity over 40 h of wakefulness. We found significant modulations over time for tests of attention (PVT), response inhibition (Go/No-Go), working memory (2-back test and addition task) and emotional processing (negative affect test; see Table 2 below). This modulation over time during 40 h consisted out of peak performance during the day, worst performance at night and a partial recovery of performance during the second day. Significant modulations over time were also found for subjective and objective sleepiness (in all 0.5 Hz bins of the wake EEG; see Table 2 below). The participants showed the highest subjective sleepiness and objective sleepiness (i.e., EEG-derived brain activity) during nighttime but became more alert again during the second day even without having slept (see also Figure 2 and 3 of the publication). The most difficult executive functioning (i.e., 3-back

test, abstract reasoning) did not show a significant modulation over time during the 40 h ($p > 0.336$).

Of particular interest in this study was a time window in the evening during the 3 h until the time of DLMO (≈2.5 h before bedtime), which is called the wake maintenance zone (WMZ) or forbidden zone of sleep (Lavie, 1986; Dijk and Czeisler, 1994). Our results showed that cognitive performance and objective sleepiness significantly improved during both WMZs compared to the prior hour (see Table 3 below; and see Figure 3 of the publication). The alerting effect of the WMZ observed in the wake EEG was especially obvious during the second evening in the constant routine, when participants were maximally sleep deprived (awake for 37.5 h). The *Body Time Study* also showed that cognitive performance and EEG brain activity returned to well rested levels in the morning after a recovery night during polychromatic bright light exposure.

Circadian Modulation over 40 h		**F and p-values**
Cognitive Performance	PVT	$F_{1,20} = 4.042$, $p < 0.001$
	Go/No-Go	$F_{1,20} = 4.815$, $p < 0.001$
	2-back test	$F_{1,20} = 3.519$, $p < 0.001$
	Addition task	$F_{1,20} = 3.611$, $p < 0.001$
	Negative affect Test	$F_{1,20} = 2.810$, $p < 0.001$
Subjective Sleepiness	KSS	$F_{1,41} = 8.596$, $p < 0.001$
EEG Brain Activity	0.5 – 25 Hz	$p < 0.001$

Table 2. Main results of the Body Time Study; 40 h time course. The *Body Time Study* showed a circadian modulation over the 40 h of wakefulness for several cognitive performance tests (n = 12), subjective sleepiness (KSS = Karolinska Sleepiness Score; n = 12) and EEG-derived brain activity (i.e., power density of the wake EEG in all 0.5 Hz frequency bins within the range of 0.5 to 25 Hz; n = 10).

Alerting Effect during the WMZ on the First Evening		**F and p-values**
Cognitive Performance	Go/No-Go	$F_{1,12} = 5.142$, $p = 0.043$
Subjective Sleepiness	KSS	ns
EEG Brain Activity	Delta & theta 3.0 – 7.0 Hz	$F_{1,38} = 4.519$, $p = 0.040$
Alerting Effect during the WMZ on the Second Evening		**F and p-values**
Cognitive Performance	PVT	$p = 0.012$
Subjective Sleepiness	KSS	ns
EEG Brain Activity	Delta & theta 4.0 – 5.0 Hz	$F_{1,38} = 11.901$, $p = 0.001$
	Alpha 10.0 – 14.0 Hz	$F_{1,35} = 5.900$, $p = 0.020$

Table 3. Main results of the Body Time Study; Wake Maintenance Zone. The *Body Time Study* showed an alerting effect of the Wake Maintenance Zone (WMZ) for two cognitive tests (n = 12) and EEG frequency ranges (n = 10), but not for subjective sleepiness (KSS; ns = not significant; n = 12). *Upper half of the table:* during the WMZ of the first evening of the constant routine there was a significant improvement in the performance on the Go/No-Go test as well as a significant reduction of power density in the lower EEG frequency ranges (i.e., delta & theta). *Lower half of the table:* during the

WMZ of the second evening of the constant routine there was a significant improvement in the performance on the Psychomotor Vigilance Test (PVT) as well as a significant reduction of power density in the EEG delta, theta, and alpha frequency ranges. A Wilcoxon signed-rank test was performed on the not normally distributed PVT data.

3.3 Study 3: The Daytime Lighting Study

Participants who were exposed to 3 h of low intensity lighting conditions (i.e., 100 photopic lux) showed a significant alerting effect in objective sleepiness (i.e., AAT Index and EEG alpha activity) dependent on the light spectrum (i.e., melanopsin activation; see upper part of Table 4 below; and see Figure 5 of the publication). Alertness was significantly increased by the high-mel and highest-mel lighting conditions compared to the low-mel condition or dim light. Participants who were exposed to higher intensity lighting conditions (i.e., 200, 600 and 1200 photopic lux) showed no significant differences in objective sleepiness (AAT Index) between lighting conditions of different spectral power distribution or photopic light intensity (see lower part of Table 4 below). Apart from the frequency ranges well known for objective sleepiness we did find significant reductions in other EEG frequency ranges, namely EEG sigma and beta, depending on light spectrum and photopic light intensity (see also Figure 6 of the publication).

Subjective sleepiness was not significantly affected by the different spectral compositions in low intensity lighting conditions but did show an acute alerting effect (after 20 min of light exposure) in higher intensity lighting conditions depending on light spectrum ($p = 0.045$; see also Figure 4 of the publication).

The pupillary light response showed significant effects in low as well as in higher intensity lighting conditions, depending on spectral power distribution and photopic light intensity (see Table 5 below; and see Figure 8 of the publication). The pupillary light response was smaller (i.e., faster redilation of the pupil) with higher melanopic irradiance or higher photopic light intensity. The sensitivity of the pupillary light response and its ability to differentiate between relatively similar lighting conditions was used to produce dose-response curves for the effect of light exposure on the pupillary light response (see also Figure 10 of the publication).

In Low Illuminance		**F and p-values**
Higher Melanopic Irradiance	Higher AAT Index Lower EEG alpha activity	$F_{3,72} = 5.452$, $p = 0.002$ $p < 0.05$
In Higher Illuminance		**F and p-values**
Higher Melanopic Irradiance	No effect on AAT Index No effect on EEG delta, theta or alpha activity	$F_{1,99} = 0.118$, $p = 0.732$ ns
Higher Photopic Light Intensity	No effect on AAT Index Lower EEG theta and alpha activity	$F_{2,125} = 0.295$, $p = 0.745$ $p < 0.05$

Table 4. Main results of the Daytime Lighting Study; objective sleepiness. This table shows the main results of the *Daytime Lighting Study* in objective sleepiness. *Upper part of the table:* in low illuminance conditions (n = 24) there was a significant effect of melanopic irradiance (column 1), with higher compared to lower melanopic irradiance leading to an increased objective alertness (i.e., increased AAT index and reduced power density in the EEG delta, theta, and alpha frequency ranges). Column 2 shows in which specific measures these alerting effects were found with the corresponding F and p-values listed in column 3. *Lower part of the table:* in higher illuminance conditions (n = 48) the spectral power distribution and photopic light intensity did not affect the main measure for objective alertness (AAT Index) but did reduce EEG theta and alpha activity. AAT Index = Alpha Attenuation Index. EEG = Electroencephalography. ns = not significant.

In Low Illuminance		**F and p-values**
Higher Melanopic Irradiance	Reduced max CA Reduced PIPR	$F_{3,255} = 15.759$, $p < 0.001$ $F_{3,263} = 26.348$, $p < 0.001$
In Higher Illuminance		**F and p-values**
Higher Melanopic Irradiance	Reduced max CA No effect on PIPR	$F_{1,438} = 5.490$, $p = 0.020$ $F_{1,190} = 2.375$, $p = 0.125$
Higher Photopic Light Intensity	Reduced max CA Reduced PIPR	$F_{2,527} = 17.678$, $p < 0.001$ $F_{2,523} = 11.937$, $p < 0.001$

Table 5. Main results of the Daytime Lighting Study; pupillometry. This table shows the main results of the *Daytime Lighting Study* in pupillometry. *Upper part of the table:* in low intensity lighting conditions (n = 24) there was a significant effect of spectral power distribution (column 1), with higher compared to lower melanopic irradiance leading to a reduced pupillary light response. Column 2 shows in which specific measures these effects were found with the corresponding F and p-values listed in column 3. *Lower part of the table:* in higher intensity lighting conditions (n = 48) there was a significant effect of light spectrum and photopic light intensity, with higher melanopic irradiance or photopic light intensity leading to a reduced pupillary light response. Max CA = maximum Contraction Amplitude. PIPR = Post-Illumination Pupil Response.

4.0 Discussion

The three main studies of this dissertation showed that by manipulating lighting conditions, especially the spectral power distribution, it may be possible to acutely impact alertness and cognitive function during daytime and nighttime. In the *Nightshift Study* we showed that filtering out short-wavelength light during nighttime protected against the negative effects of light exposure during isolated nightshifts by not suppressing or shifting the melatonin profile, while at the same time acutely improving reaction time (compared to dim light). In the *Body Time Study* we confirmed that during prolonged wakefulness and in the absence of light, cognition and alertness (i.e., EEG-derived brain activity) showed not just a homeostatic decline but also a circadian modulation under control of the endogenous biological clock, which resulted in increased alertness during the WMZ in the evening. At the time in the morning when these circadian rhythms of cognition and alertness had shown a peak during the *Body Time Study* (3 h after waking), we showed in the *Daytime Lighting Study* that in well-rested participants manipulation of the light spectrum could increase alertness even further. This improved daytime alertness was especially evident when light spectrum was manipulated at low light intensities and depended on melanopic irradiance. The pupillary light response was found to be sensitive to differences in light spectrum at low and high light intensities and showed a dose response curve with melanopic irradiance during daytime. This dose response curve demonstrates the potential of the implementation of the pupillary light response as an objective marker of how daytime lighting affects physiology.

4.1 Shift work

The importance of telling internal time was recently acknowledged by the Nobel Prize in Physiology and Medicine (2017), which was awarded to the researchers who uncovered the molecular machinery of the biological clock in fruit flies. This molecular clock can be found in every cell of the human body. Each cell would be ticking to its own rhythm if it were not for the central biological clock in the brain (i.e., the SCN). The SCN synchronizes all the different circadian rhythms of cells and tissues with the outside light-dark environment. If this central control is deregulated, for example during nightshifts by light exposure at a time when it should be dark, this has repercussions for many aspects of health. Studies have shown that aberrant light dark patterns, especially as a result of nightshifts, can lead to an increased risk of cancer, cardiovascular diseases, weaker immune responses, sleep problems, obesity, diabetes and depression [for a review see: (Wang et al., 2011)] . Yet, the number of people who work outside of the regular daytime office hours is increasing, with 21% of workers in the European Union performing shift work in 2015 (Eurofound; Sixth European Working Conditions Survey; 2017 update). A lot of research has been performed on how to improve conditions during nightshifts but so far this has only been able to partially mitigate the negative health effects in shift workers. Previous studies have mainly focused on consecutive nightshifts with the aim to shift people's biological clock to fit with their work schedule of being awake at night and asleep during the day. However, even if people work nightshifts during weekdays, they want to socialize with their friends and family in the weekend, shifting them back to diurnal activity patterns. Since it takes days for people to shift their rhythms fully from diurnal to nocturnal, or vice versa, these people would be out of synchrony the entire time, the internal time never running synchronous with their sleep-wake rhythm. They would be living with a constant jetlag of a few hours. Therefore, completely shifting the endogenous clock is only to be recommended on very rare occasions.

Especially in the case of isolated nightshifts no phase shifting at all is desired since people will have to go back to their diurnal rhythm immediately after the nightshift. Exposure to regular lighting during the nightshift would shift their clock. Exposure to short-wavelength light (which is included in all white lighting conditions) in the night or early morning, shifts the biological clock to a later or earlier time respectively (Khalsa et al., 2003). While previous studies had already shown that filtering out the short-wavelengths of light could prevent or greatly reduce melatonin suppression they did not investigate phase shifts (see also Table 1 of the *Nightshift Study* for an overview of these studies). The first study of this dissertation (i.e., the *Nightshift Study*) showed to the best of our knowledge for the first time that filtering out the short-wavelength light during an isolated nightshift prevented phase shifting, compared to a dim light condition.

In order to measure internal time and phase shifts the *Nightshift Study* participants had to remain in dim light conditions for several hours in the evening so that melatonin concentrations in saliva samples could be determined. This method to determine internal time is quite cumbersome. Therefore, it is not often used outside of the laboratory, even though it has been shown that many drugs or medical interventions have a higher chance of success when given at the right internal time (Zhang et al., 2014). The *Body Time Study* was part of a collaboration that sought to change this by developing a tool that can determine internal time with one blood sample [see co-authored study: (Wittenbrink et al., 2018)]. This tool may be of great help in future research on circadian rhythms and have many clinical applications.

The ability of nighttime lighting conditions to boost alertness may be just as crucial during nightshifts as efforts to protect against the negative health effects of melatonin suppression and phase shifts. Associations have been found between shiftwork and accidents, either at the workplace or during transit after working nightshifts. Part of the alerting response of light at night has been shown to occur concomitant with melatonin suppression (Cajochen et al., 2000). Melatonin is suppressed by light, especially by short-wavelength light, as a dose response curve for melanopic light intensity and melatonin concentration showed during the evening [see co-authored study: (Nowozin et al., 2017)]. But studies have shown that also in the absence of melatonin suppression nighttime lighting can boost alertness (Rahman et al., 2011). The *Nightshift Study* confirmed this by finding a significant improvement in cognition when compared to dim light, while melatonin was not significantly suppressed by the filtered lighting condition. This shows that manipulating the light spectrum could help protect the health of shift workers, while keeping them alert during nighttime. One caveat might be that filtering out the short-wavelength light resulted in an orange colored lighting, which caused low color rendering. Therefore, it may not be recommended for work environments where a perfect ability to distinguish colors is crucial (e.g., surgery).

4.2 Sleep deprivation

Although some shift workers may have the opportunity to get some sleep during their shifts or are able to take naps immediately prior to a nightshift, they will be awake for a prolonged period of time and may have trouble getting sufficient sleep afterwards. Many of them therefore experience sleep deprivation and accumulated sleep depth. Experiencing such frequent sleep deprivation is not limited to this population group. Many people use daily alarm clocks to wake up in the morning, cutting their sleep short below the recommended 7-8 h. Those 7 or more hours are the recommended amount of sleep, with most people needing between 6 to 10 h (Watson et al., 2015). Socializing in the evening and fixed work times in the morning make people restrict their sleep duration. During the weekends when they do not

have to wake up for work many people sleep in (especially late chronotypes), shifting themselves to later times. Then on Monday they have to shift back to wake up early for work. The resulting mismatch between internal and external time is called ‘social jetlag’ and a greater social jetlag has been associated with negative health effects like a higher body weight, chance of obesity and a higher probability of smoking (Roenneberg et al., 2012). People often try to negate their sleep debt that accumulates over the workweek, for example with caffeine intake. Caffeine has alerting effects because it has an antagonistic effect on the adenosine receptors in the brain. Adenosine builds up in the brain with extended duration of wakefulness and is usually flushed out during sleep [for a review on caffeine effects see (McLellan et al., 2016)]. So, although caffeine may help to stay alert it only blocks the sleepiness signal but does not affect the underlying cause of sleepiness and does nothing to protect against the negative health effects of insufficient sleep. Also, studies have shown that it is not possible to fully catch up on lost weekday sleep during the weekends (Depner et al., 2019). It has been shown that chronic sleep restriction of even just two hours (i.e., fourteen nights of 6 h allowed for sleep instead of 8 h) can impair neurobehavioral functioning (Van Dongen et al., 2003). The sleep debt was shown to accumulate over fourteen days to reach levels similar to two nights of total sleep deprivation (i.e., skipping entire nights of sleep). More long-term studies are needed to determine how moderate, but chronic, sleep restriction over decades affects health.

Effects of acute sleep deprivation are easier to investigate, although also here longer lasting effects have been found. Several studies have shown detrimental effects of acute sleep deprivation that lasted even beyond multiple recovery nights (Belenky et al., 2003). In the recovery after acute sleep deprivation, lighting conditions could be beneficial. The *Body Time Study* showed that when sleep deprived participants were exposed to polychromatic bright lighting during the morning, after only one 8-h recovery night, their cognition and alertness returned to well-rested baseline levels. Whether this recovery lasted beyond the duration of morning light exposure and whether this was not just masking the effects of sleep deprivation, needs to be investigated in further studies. One limitation of the *Body Time Study* was that it did not have a control condition without bright light exposure in the morning after the recovery night. Therefore, the results in the morning could only be compared to that of previous studies, which have found that just one recovery night is insufficient for a full recovery.

The *Body Time Study* also showed an interaction between the circadian and homeostatic influence on alertness. According to the 2-process model of sleep regulation (Borbely, 1982) a homeostatic component of sleepiness increases the longer someone is awake until it is relieved by sleep. A second component, the circadian component, induces a 24-h modulation of sleepiness. When a person is synchronized to the outside light-dark environment and not sleep deprived the interaction between these two components results in a consolidated wake period of about 16 h during the day and a sleep episode of about 8 h at night. A modified version of the 2-process model [(Bes et al., 2009) using the rhythm of REM sleep occurrence as its circadian component] shows that the interaction of the two components results in high alertness in the morning (i.e., low homeostatic sleepiness and high circadian sleepiness), the post lunch dip in the afternoon (i.e., intermediate homeostatic sleepiness and intermediate circadian sleepiness), the WMZ in the evening (i.e., high homeostatic sleepiness but low circadian sleepiness), and sleep at night (i.e., high homeostatic sleepiness and high circadian sleepiness). In the *Body Time Study* we confirmed what previous studies have shown that the slower EEG frequency ranges of delta (0 – 4 Hz) and theta (4 – 8 Hz) activity, as well as the faster EEG frequency ranges of sigma (13 – 15 Hz) and beta (15 – 25 Hz) activity, showed a mostly homeostatic increase in power density over 40 h of wakefulness.

Higher EEG power density in the low frequency ranges means a higher objective sleepiness (Finelli et al., 2000). The EEG alpha frequency range (8 – 13 Hz) on the other hand has previously been found to show a circadian rhythm (Cajochen et al., 1999). In the *Body Time Study* we also showed a strong circadian influence in EEG alpha activity modulation over 40 h of wakefulness, with peak power density in the afternoon and lowest power density in the night to early morning. Yet, all EEG frequency ranges were to some extent affected by both the homeostatic and circadian component. We showed that during the second WMZ after participants had been awake for about 37.5 h the EEG delta, theta, sigma and beta frequency ranges showed a reduction in power density. During the first WMZ (i.e., after about 12 h of wakefulness) there had been no significant reduction in power density for these frequency ranges compared to the prior hour. The circadian influence therefore only became apparent when sleep pressure was very high after one night of sleep deprivation. Likewise, we showed that EEG alpha activity was not solely affected by the circadian component but also by the homeostatic component, as indicated by a higher peak of power density during the second day compared to the first day of the 40 h of sleep deprivation. The resolution of measurements in the *Body Time Study* was relatively high (i.e., hourly measures) compared to previous constant routine studies which enabled this detailed analysis of the modulation of alertness over time.

4.3 Daytime alerting effects of light exposure

Previous studies have indicated that especially the morning may be an important moment for light exposure to improve cognition and alertness, with effects persisting until much later during the day [see co-authored study: (Münch et al., 2017)]. Blue-enriched morning light was shown to reduce phase shifts caused by bright light exposure in the evening. Based on these results, the time window of the morning to early afternoon was chosen in the *Daytime Lighting Study* as the optimal time to investigate the effects of light exposure. The start of the light exposure was about 2.5 h after waking to avoid interference from sleep inertia, which is the phenomenon of feeling even more tired directly after waking than in the evening before bedtime. It usually lasts one or two hours. The aim of the *Daytime Lighting Study*, however, was to determine in well-rested individuals what the optimal daytime lighting condition is. Therefore, light exposure started in the late morning after sleep inertia had dissipated.

Findings from previous studies have shown different results concerning the alerting effects of light exposure during daytime. Some studies did find daytime alerting effects while others did not [for a review see (Lok et al., 2018)]. While previous studies have investigated either light intensity or light spectrum concerning daytime alerting effects, the *Daytime Lighting Study* was to the best of our knowledge the first to investigate both a range of light intensities (i.e., photopic light intensities) and light spectra (i.e., melanopic irradiances) in one study protocol. The hypothesis was that higher light intensity would result in higher alertness and that higher melanopic irradiance (i.e., higher proportion of short-wavelength light) would result in higher alertness. This would mean that the highest light intensity that had the highest melanopic irradiance would result in the highest alertness. However, this was not the case. Objective sleepiness, as measured in the wake EEG, showed that the participants indeed had a higher alertness in the light spectrum condition with the highest melanopic irradiance, but only at low light intensities of a 100 photopic lux. At this quite low light intensity (e.g., a sunny day outside is between 50.000 and 100.000 photopic lux), objective sleepiness was affected by different light spectrum conditions as hypothesized. Yet, when light intensity was increased to 200, 600 or 1200 photopic lux no significant differences between light spectra or light intensities were found for objective sleepiness as determined in

the EEG alpha activity range, for which a validated measure of alertness has been established [the Aplha Attenuation Test index; (Stampi et al., 1995)]. A possible explanation for this might be a ceiling effect. It could be that the higher light intensity conditions were so bright that an optimization of the light spectrum had no effect. Yet, this would mean that the ceiling lies already somewhere below 200 photopic lux. This is quite a low light intensity, seen as humans evolved in more than a hundred times brighter natural light.

In contrast to the EEG alpha activity, the *Daytime Lighting Study* did show a significant reduction of EEG beta activity based on photopic light intensity. EEG beta activity during waking has been associated with attention and cognitive performance (Barry et al., 2007). It is also important to note that some of the significant reductions in EEG power density found in the *Daytime Lighting Study* depended on the electrode derivation, i.e. brain tomography. While EEG alpha activity was reduced in all derivations, the EEG beta activity was only reduced in the frontal derivation by higher light intensities compared to lower light intensities. Since the frontal cortex is the main target region for higher executive functions, this indicates a better performance in higher light intensities. Although cognitive performance was measured in the *Daytime Lighting Study* this data has not yet been fully analyzed and will be reported separately to this dissertation.

These findings show that the association between melanopic irradiance and objective alertness is not as simple as a linear relationship. The next paragraph discusses a hypothesis on why we found the clearest effect in low intensity lighting.

4.4 Living in biological darkness

Previous studies have worked under the hypothesis that during the day more light is better, inspired by the brightness of natural daylight. The results of the *Daytime Lighting Study* showed that it may not be that simple. And in fact, results from other studies also indicate this may not be the case as many studies found no alerting effect of higher light intensities [for a review see (Lok et al., 2018)]. Many of those studies investigated alertness in cognitive performance, slow eye movements and subjective sleepiness measures. Perhaps those methods of determining alertness are not sensitive enough to detect daytime alerting effects. In the *Daytime Lighting Study* we showed that the wake EEG may be a more sensitive objective measurement for alertness. Previous studies that also used EEG measures found inconclusive results as well, with some finding significant alerting effects during daytime like the *Daytime Lighting Study* while others did not (Daurat et al., 1993; Rahman et al., 2014).

Because of the negative findings of previous studies the hypothesis may be proposed that even brighter light is needed during daytime to elicit alerting effects. Our interpretation of the results of the *Daytime Lighting Study* partly agrees with this hypothesis. Since humans evolved under natural daylight of many thousands of photopic lux it is logical to hypothesize that the best daytime lighting condition should mimic the outside natural light environment. However, most people no longer live outside and their physiology may not be used to such high intensity light exposure. In fact, studies have found that in modern society people spend more than 60% of their waking day in lighting conditions below 100 lux (Scheuermaier et al., 2010). Therefore, there may be an adaptation effect. The reason why the physiology of the participants in the *Daytime Lighting Study* was significantly differentially affected by different light spectrum conditions at low intensities but showed less effects at high intensities, may be because of the light exposure they received in the days prior to the experiment. Indeed, previous studies have shown that light history is an important factor that modulates non-visual light effects (Hébert et al., 2002). Light history can be viewed as the few hours prior to

an experimental light exposure, it can mean the few days prior to an experiment or it could be viewed as an even more long term effect of weeks, months or seasons. For example a study in the Antarctic showed that the pupil sensitivity to light took several weeks, after a long dark Antarctic winter without any natural daylight, to return to the level it was before the start of the winter (Kawasaki et al., 2018). Most previous studies that investigated daytime alerting effects have not taken into account prior light history. The *Daytime Lighting Study* also controlled the prior light history only for the two hours prior to the experiment by having participants wear dark goggles from the moment they woke up. But a habitual light exposure to low intensity lighting over a longer period may sensitize the physiology to spectral differences only at these low light intensities. This could explain why many studies found no alerting effect of bright light during daytime. The Meta title of the *Daytime Lighting Study* (i.e., "Living in biological darkness") refers to this hypothesis that in modern society people's physiology may be habituated to low indoor lighting conditions. Exposure to a few hours of high light intensity may not invoke as much of a response as an optimization of low light intensity conditions will to a physiology tuned for low light intensities. Therefore, it may be more prudent to improve the light quality of low intensity lighting conditions.

Nevertheless, to adapt our physiology to low light levels may not be healthy. The optimal lighting condition for human health may still be high intensity lighting, as long as people are exposed to these high intensity lighting conditions for most of their day and not just for short periods of time while they spend the rest of their day in low intensity lighting conditions. Future studies should investigate daytime lighting effects with participants who spent most of their waking time in higher intensity lighting during the weeks prior to the experiment. Perhaps such studies will find differential alerting effects based on light spectrum at high light intensities. But until such higher intensity lighting is more common it may be more effective to optimize the light spectrum of habitual low light intensity conditions.

4.5 Daytime markers of lighting effects

The AAT index in the wake EEG was designed as a simpler alertness measurement than the Multiple Sleep Latency Test (MSLT), which takes many hours to perform. The MSLT measures the time to fall asleep during multiple daytime naps, whereas the wake EEG takes only 5 to 10 minutes. EEG alpha activity in the wake EEG was of particular interest in the *Daytime Lighting Study* as a candidate marker for daytime light effects, because acute lighting effects on EEG alpha activity have been shown as well as a circadian variation over time (Cajochen et al., 1999). If EEG alpha activity could be used as a daytime marker of the effects that different lighting conditions have on the physiology this would help in designing better daytime lighting conditions. In the *Daytime Lighting Study* we showed for the AAT index (which is based on EEG alpha activity) an increased alertness which dependent on the melanopic irradiance of a lighting condition, but only at low light intensities (as discussed above). That EEG alpha activity is sensitive to relatively small changes in light spectra is promising and shows that the EEG is an interesting parameter when researching daytime light effects. But since we were not able to distinguish EEG alpha activity differences between different lighting conditions at higher light intensities, and because it is difficult to perform an EEG outside of a laboratory setting it may not be the perfect marker of daytime lighting effects.

In the *Daytime Lighting Study* we showed that pupillometry might be used as an even simpler measure of non-visual light effects on physiology. What makes pupillometry especially interesting is that, just like for EEG alpha activity, it has been found that the post-illumination pupil response has a circadian variation (Münch et al., 2012). To measure the

pupillary light response is much less complicated and demanding than applying the wake EEG, because a pupillometry measurement takes only 35 s and can be performed with a handheld device. It can therefore also easily be performed outside of a laboratory setting. We found that in addition to being sensitive to light spectrum (i.e., melanopic irradiance) the pupillary light response was also sensitive to different photopic light intensities. We found this for the immediate pupil response (i.e., max CA) as well as for the more sustained pupil response (i.e., PIPR). Dose response curves could be created for the pupillary light response and the alpha-opic irradiances of light. For the sustained pupil response the best dose response curve was found with melanopic irradiance, compared to all other alpha-opic irradiances. This confirmed previous studies which showed that melanopsin is especially important for the sustained pupil response. The dose response curve may be used to predict the effect of ambient lighting conditions on the physiology. This means that pupillometry could be a useful tool to test the physiological effect of new lighting designs in the future.

Being able to measure or predict the non-visual effects of a lighting condition could be beneficial for healthy people as well as for people suffering from certain diseases. As previously mentioned, light is the main information input to the circadian system. Insufficient lighting may cause the circadian phase to drift, thereby desynchronizing internal rhythms of the body with the outside environmental time. The ability to easily measure which lighting condition has the strongest non-visual effects may be of help in lighting design which aims to stabilize this synchronization of circadian rhythms. Especially during the winter, when there is less natural light, optimization of artificial light may be important. For example, bright light therapy is successfully used to treat depression, especially seasonal affective disorder [SAD; (Rosenthal et al., 1990)]. And in Alzheimer patients, who spent most of their time indoors, it was found that the installation of bright lighting in the living room of an elderly home stabilized their circadian rhythms, which was seen in a reduction of nighttime agitation (Van Someren et al., 1997).

5.0 Conclusion

In conclusion, findings summarized in this dissertation showed that a change in light spectrum during nightshifts could prevent negative health effects of phase shifts and melatonin suppression while improving cognitive performance. Objective sleepiness measured in brain activity, especially EEG alpha activity, was found to be a good marker of daytime light effects. Manipulations of light spectrum at low light intensities improved objective sleepiness during daytime, while at higher light intensities fewer differences were found between light spectrum conditions. This might be due to a long-term adaptation of living in low intensity indoor lighting conditions, which is highly prevalent in modern society. Pupillary light sensitivity was found to be an even more sensitive and less demanding objective marker of light effects during daytime, which could be used to distinguish between different photopic light intensities and different spectral lighting conditions. Development and future implementation of these objective daytime markers could help in the research and design of optimal daytime lighting conditions in order to improve people's health.

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Declaration of own work / affidavit

Statutory Declaration

“I, Jan de Zeeuw, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic ‘From darkness to light: Non-visual light effects can be modulated by optimizing light spectrum during nighttime and daytime’, independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I am aware of the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice and that I commit to comply with these regulations.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.”

Date	Signature

Declaration of contribution to any publications

Jan de Zeeuw contributed the following to the below listed publications:

Publication 1: Regente J*, de Zeeuw J*, Bes F, Nowozin C, Appelhoff S, Wahnschaffe A, Münch M#, Kunz D#., Can short-wavelength depleted bright light during single simulated night shifts prevent circadian phase shifts?, Journal of Applied Ergonomics, 2017 (* = co-first authors; # = co-last authors)

Contribution:

While performing the experiment the two first authors, Johannes Regente and Jan de Zeeuw, performed an equal number of nightshifts during which data was collected. Initially, Jan de Zeeuw analyzed the cognitive performance (data on which Figure 3 and Table 2 are based) as well as the EEG data of the sleep episodes (data on which Figure 4 is based) and not the melatonin or sleep data. However, in preparation for publication Jan de Zeeuw redesigned all

Figures and Tables and redid all the statistical analysis. Johannes Regente, Mirjam Münch and Jan de Zeeuw wrote the manuscript.

Publication 2: de Zeeuw J, Wisniewski S, Papakonstantinou A, Bes F, Wahnschaffe A, Zaleska M, Kunz D[#], Münch M[#], The alerting effect of the wake maintenance zone during 40 hours of sleep deprivation, Scientific Reports, 2018 ([#] = co-last authors)

Contribution:

Jan de Zeeuw was the study leader of the experiment which meant that he recruited the participants, and was on call during the entire experiment in case problems occurred. During data collection Jan de Zeeuw performed the nightshifts of the 40 h constant routines and some evening shifts. After the data was cleaned by co-authors, all the data analysis for this publication was performed by Jan de Zeeuw. Jan de Zeeuw designed all Figures. Jan de Zeeuw wrote the manuscript. At all stages of the data analysis and the writing of the manuscript all co-authors gave their input (see also the attached publication for the paragraph 'Author contributions').

Publication 3: de Zeeuw J, Papakonstantinou A, Nowozin C, Stotz S, Zaleska M, Hädel S, Bes F, Münch M[#], Kunz D[#], Living in biological darkness: Objective sleepiness and the pupillary light responses are affected by different metameric lighting conditions during daytime, Journal of Biological Rhythms, 2019 ([#] = co-last authors)

Contribution:

Jan de Zeeuw and four co-authors designed the experiment. Jan de Zeeuw was the study leader of the experiment and was on call during the entire experiment in case problems occurred. Jan de Zeeuw performed half of the participants' recruitment while a co-author performed the other half of the recruitment. The data collection was performed by Jan de Zeeuw and five co-authors. After the EEG data was cleaned by co-authors Jan de Zeeuw performed all data analysis. Jan de Zeeuw cleaned the pupillometry data and performed all data analysis. Jan de Zeeuw designed all Figures and Tables and wrote the manuscript. At all stages of the data analysis and the writing of the manuscript all co-authors gave their input (see also the attached publication for the paragraph 'Author contributions').

Signature, date and stamp of supervising university professor / lecturer

Signature of doctoral candidate

Copies of publications

Study 1: Nightshift Study

Johannes Regente*, Jan de Zeeuw*,
Frederik Bes, Claudia Nowozin,
Stefan Appelhoff, Amely Wahnschaffe,
Mirjam Münch, Dieter Kunz

(*co-first authors)

Applied Ergonomics 2017
Volume 61, Page 22-30
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Study 2: Body Time Study

Jan de Zeeuw, Sophia Wisniewski, Alexandra Papakonstantinou, Frederik Bes, Amely Wahnschaffe, Mandy Zaleska, Dieter Kunz, Mirjam Münch

Scientific Reports 2018
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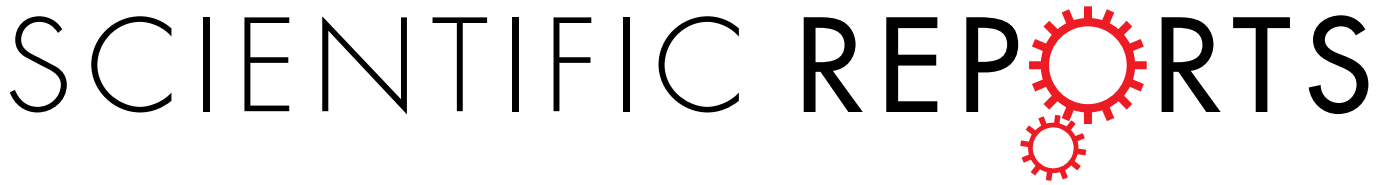

OPEN

The alerting effect of the wake maintenance zone during 40 hours of sleep deprivation

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Under entrained conditions, the accumulation of homeostatic sleep pressure in the evening is opposed by a strong circadian arousal signal prior to the dim light melatonin onset, called the Wake Maintenance Zone (WMZ). This study aimed at investigating the impact of the WMZ on different cognitive performance tests, as well as on subjective and objective sleepiness. Twelve young male participants completed a constant routine protocol with 40 h of extended wakefulness that included two WMZs. Cognitive tests and saliva samples were assessed hourly, while the electroencephalogram (EEG) was recorded continuously. Participants improved in cognitive response inhibition during WMZ1 (13.5 h awake) and sustained attention during WMZ2 (37.5 h awake), but not in higher executive function tests. There were significant EEG power density reductions in the delta/theta frequency range during WMZ1 and in delta/theta, alpha, and sigma/beta ranges during WMZ2, with a greater change in the sigma/beta range during WMZ2 compared to WMZ1. EEG power reductions coincided during WMZ1 with stable subjective sleepiness and sustained attention. During WMZ2, EEG power reductions were more pronounced and coincided with improved sustained attention. Our results suggest the circadian arousal signal in the evening differently modulates cognitive functions and EEG power depending on the duration of prior wakefulness.

Mammalian sleep-wake regulation undergoes modulation by two main processes. A homeostatic process (Process S) accounts for the build-up of sleep pressure, i.e. it increases with time awake. This can be assessed by the increase of slow frequency EEG activity during wakefulness and its decline during the following sleep period[1–4]. However, sleep pressure does not linearly increase over time[5], even after extended wakefulness by partial or total sleep deprivation (as reviewed by Schmidt *et al.*[6]). Instead, sleep propensity (the tendency to fall asleep) is also modulated by a circadian process. The circadian component (Process C) regulates the endogenous circadian rhythm of sleep-wake regulation across 24 hours[1] and is governed by the biological clock in the suprachiasmatic nucleus of the hypothalamus (SCN)[7]. Both processes interact with each other and can only be determined separately by means of specialized protocols such as the forced desynchrony protocol[8] or nap paradigms[9]. When analyzing the circadian impact on sleep propensity under these conditions, the circadian drive for sleep is very low in the early evening before bedtime, shortly before the secretion onset of the pineal hormone melatonin[8]. At first glance, this may seem counterintuitive, because it implies a high circadian drive for alertness in the evening[8–12]. Yet, it was shown that the circadian arousal signal in the early evening leads to higher subjective and objective alertness and is thus opposing the accumulated homeostatic sleep pressure - a phenomenon called the 'wake maintenance zone' (WMZ) or 'forbidden zone of sleep'[5,9,11]. The dynamic interaction of both processes in sleep-wake regulation enables a consolidated wake period of approximately 16 hours during daytime and a consolidated sleep period at night in humans[13,14].

In addition to sleep-wake regulation, most physiological and behavioral processes undergo homeostatic and circadian regulation. Cognitive performance, as well as subjective and objective alertness, is also high during the

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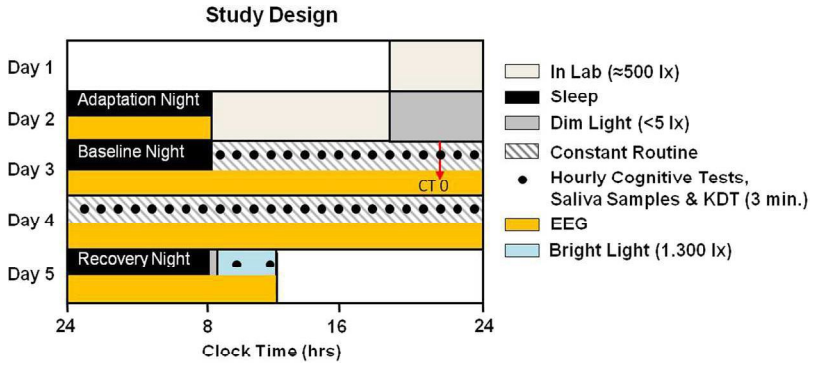

Figure 1. Study Design. Participants arrived at the laboratory 3 h prior to habitual bedtime on day 1 and slept in the laboratory at their habitual bed- and wake-times (=adaptation night). On day 2 participants stayed in the laboratory where they were free to move in their room. Following the baseline night, the 40 h CR protocol with constant wakefulness in bed started at habitual wake time on day 3. During the CR, cognitive tests and Karolinska Drowsiness Tests (KDTs; 3 min; open eyes) were performed hourly. Salivary samples for hormonal analyses were also collected hourly. The first cognitive tests were performed at CT -13, which was 13 h prior to the DLMO of the first evening of the CR (DLMO = CT 0; red arrow). The CR protocol ended at CT 26. After the recovery night on day 5 participants were exposed to 3 h of bright light (for purposes not reported here) and were free to leave the laboratory about 4 h after habitual wake time.

WMZ in the evening[15–24]. Significant performance improvements of sustained attention occur during the WMZ, both after a normal duration of prior wakefulness as well as after sleep deprivation[16]. Better working memory performance during the WMZ has been linked to sleep-dependent higher hypothalamic activation as assessed in the Blood Oxygenation Level Dependent (BOLD) response of the MRI after a normal duration of prior wakefulness, but not after very low or very high sleep pressure conditions[25]. These studies suggest a differential impact on cognitive performance during the WMZ, depending on the duration of prior wakefulness.

We aimed at measuring different domains of cognitive performance, such as sustained attention, executive functions and working memory, as well as subjective and objective sleepiness during 40 hours of extended wakefulness and under controlled conditions (i.e. constant routine[26]; CR). The experiment was designed to include two WMZs. We hypothesized that not all cognitive functions are similarly altered over the course of the protocol and specifically not during the two WMZs. In addition, we expected differences in subjective and objective sleepiness between both WMZs, which depend on the prior duration of wakefulness.

Results

Cognitive performance. *Time course of cognitive performance during 40 hours of extended wakefulness.* The time course of all results was expressed as circadian time (CT) relative to the individual timing of the dim light melatonin onset (DLMO) in the first evening of the CR (= CT 0; see also Fig. 1 and Methods) which occurred on average at 21:17 (±1:09 h; SD). For the time course across 40 h of extended wakefulness there was a significant modulation in all but three cognitive tests (Psychomotor Vigilance Test (PVT) median reaction times: $F_{1,20} = 4.042$, $p < 0.001$; PVT lapses: $F_{1,20} = 3.208$, $p < 0.001$; Go/No-Go: $F_{1,20} = 4.815$, $p < 0.001$; 2-back: $F_{1,20} = 3.519$, $p < 0.001$; Addition Task: $F_{1,20} = 3.611$, $p < 0.001$; Negative Affect: $F_{1,20} = 2.810$, $p < 0.001$; main effects of TIME; Fig. 2a–c,e,g; Table S1.1). Post-hoc comparisons between time points for each test (corrected for multiple comparisons, see Methods) revealed a very similar time course for the PVT, Go/No-Go and the Addition Task with best performance during the first biological day (i.e. CT −13 until CT 0; Fig. 2a–c and e). Immediately following the DLMO (i.e. CT 0), performance on the PVT, Go/No-Go, and Addition Task gradually declined, leading to significantly lower levels during the late biological night/early morning (CT 7 until CT 13), when compared to the first biological day. Compared to the late night/early morning, performance significantly improved during the second day (starting at CT 15). Results for the 2-Back test showed a similar time course, except that cognitive performance on the first day remained at high levels for longer (until CT 5; Fig. 2c). The more difficult version of the N-Back test (3-Back) showed no significant change over time; neither did performance in the Word-Memory Test or the Abstract Reasoning Test (3-Back: $F_{1,20} = 1.124$, $p = 0.336$; Word-Memory: $F_{1,20} = 1.109$, $p = 0.349$; Abstract Reasoning: $F_{1,20} = 0.789$, $p = 0.722$; Fig. 2c,d and f).

The time course for correctly recognized negative emotions (=Negative Affect Test) showed a significant modulation that differed from the other tests ($F_{1,20} = 2.810$, $p < 0.001$; Fig. 2g). Here, performance was high at the very start of the CR (at CT −13) and declined during the morning and early afternoon. In the late afternoon (CT −5), performance improved and remained high until the evening (CT 1). It then decreased again and, compared to the time between CT −5 and CT 1, performance remained significantly lower for the rest of the CR.

Cognitive performance changes during the two wake maintenance zones. The two WMZs were defined as the 3-hour interval prior to DLMO. We analyzed the changes during the WMZs in three ways (see also Fig. S1): First, we compared absolute performance levels between the hours directly prior to the WMZs (PH 1 vs. PH 2) and we compared the absolute performance levels during the two WMZs with each other (WMZ 1 vs. WMZ 2). Secondly, we compared the performance during each WMZ with the hour preceding it (WMZ 1 vs. PH 1; and WMZ 2 vs.

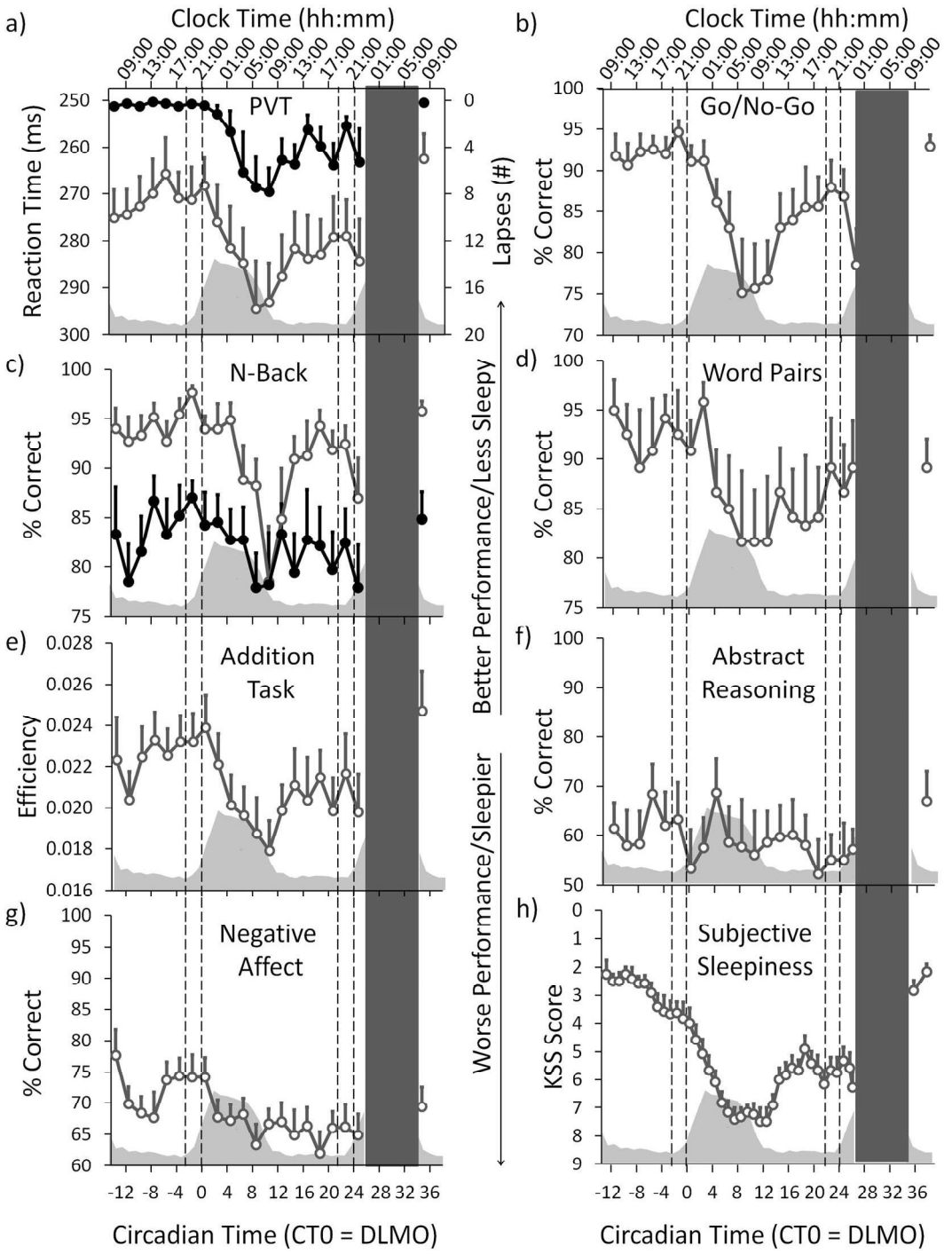

Figure 2. Cognitive performance and subjective sleepiness. The upper x-axis shows the mean clock time. The lower x-axis shows the circadian time in hours relative to the DLMO of the first evening (= CT 0). Note: the y-axis for PVT performance and the KSS have been inverted so that the direction is similar to the other cognitive tests (i.e. higher indicates better performance and less sleepy). The grey inlay shows the melatonin secretion profile across the CR and during the first 4 h after the recovery night. The two sets of dotted lines indicate the time ranges of both WMZs. The black bar represents the scheduled sleep episode (=recovery night). Mean ± SEM (n = 12). See also Table S1.1. (**a**) PVT; open grey circles = median reaction times; filled circles = lapses. (**b**) Go/No-Go Test. (**c**) N-back Test; open grey circles = 2-Back version; filled circles = 3-Back version (ns). (**d**) Word-Pair Memory Test (ns). (**e**) Addition Task. (**f**) Abstract Reasoning (ns). (**g**) Negative Affect Test. (**h**) Subjective Sleepiness (KSS).

PH 2). Lastly, we determined whether extended wakefulness had a differential impact on performance changes during the WMZs by comparing the changes in performance between the two WMZs (i.e. while each WMZ was expressed relative to its prior hour; WMZ 1/PH 1 vs. WMZ 2/PH 2).

Comparison of the absolute performance levels between the two WMZs showed significantly more PVT lapses ($p = 0.047$, Cohen's d effect size = 0.968) as well as less accuracy on the Go/No-Go ($F_{1,11} = 7.084$, $p = 0.022$, $d = -0.747$) and the 2-Back Test ($F_{1,16} = 13.921$, $p = 0.002$, $d = -1.160$) during the second WMZ (=WMZ 2; after 37.5 h awake) compared to the first WMZ (=WMZ 1; after 13.5 h awake; Fig. 2a–c). The other performance tests did not show significant differences between both WMZs. In the hour prior to WMZ 2, performance was significantly worse compared to the hour prior to WMZ 1 for PVT lapses, the Go/No-Go, the 3-Back Test, the Word-Memory Test and the Addition Task (respectively: $p = 0.008$, $d = 1.108$; $F_{1,10} = 6.774$, $p = 0.027$, $d = -0.697$; $F_{1,13} = 8.180$, $p = 0.014$, $d = -0.804$; $F_{1,11} = 10.729$, $p = 0.007$, $d = -1.135$; Fig. 2a–e; Table S1.2.1).

Comparing the performance during each WMZ with the hour immediately prior to the respective WMZ, we found that during WMZ 1 (i.e. at CT −2) the Go/No-Go was the only cognitive test which showed a significant performance improvement compared to the test taken prior to WMZ 1 (i.e. CT −4; $F_{1,12} = 5.142$, $p = 0.043$, $d = 0.380$; Fig. 2b). During WMZ 2 (i.e. CT 23) there were significantly less PVT lapses compared to the test taken prior to WMZ 2 (i.e. CT 21; $p = 0.012$, $d = -0.687$; Fig. 2a; Table S1.2.2).

Then, we calculated and compared the cognitive performance changes of the two WMZs (with each WMZ was expressed relative to its prior hour). This showed a significant greater decrease in PVT lapses ($p = 0.009$, $d = -0.986$) and a greater increase of efficiency in the Addition Task ($F_{1,11} = 6.843$, $p = 0.025$, $d = 0.808$) during WMZ 2, when compared to WMZ 1 (Table S1.2.3). The changes in other performance tests did not show significant differences between both WMZs.

Cognitive performance after the recovery night. After the 8 h recovery night and during the polychromatic bright light exposure (see Methods), cognitive performance improved again to levels similar to those at the beginning of the CR protocol for all tests, except for PVT median reaction times: these were significantly faster than during the first 6 h of the CR ($p < 0.05$; Fig. 2a–g).

Subjective and objective sleepiness. *Time course of subjective and objective sleepiness across 40 hours of extended wakefulness.* Subjective sleepiness showed a significant change over time (main effect of TIME; $F_{1,41} = 8.596$, $p < 0.001$; Fig. 2h; Table S2.1). Post-hoc tests showed that participants rated themselves least sleepy during the first morning and early afternoon of the CR (CT −13 until CT −7). Compared to the morning and early afternoon, participants were significantly sleepier in the late afternoon (starting at CT −5) and became most sleepy in the late night/early morning hours (CT 7 until CT 12). During the second day, participants were significantly less sleepy (starting at CT 15) compared to the late night/early morning. However, compared to the first day (CT −13 until CT 0), participants remained significantly sleepier during the entire second day (i.e. until CT 26; $p < 0.05$).

For objective sleepiness, the waking EEG power density of the 3 min Karolinska Drowsiness Test (KDT; see Methods) showed a significant change over time for all 0.5 Hz bins in the range of 0.5–25 Hz ($p < 0.001$; Fig. 3a). Here, we report the results of the frontal derivation (F4), but similar results were found in central and occipital derivations (data not shown). Special interest was given to those EEG frequency ranges which showed a significant change in EEG power density during the WMZs (EEG delta/theta 4–5 Hz: $F_{41,232} = 8.596$, $p < 0.001$; alpha 10–14 Hz: $F_{41,208} = 2.861$, $p < 0.001$; and sigma/beta activity 15.5–23 Hz: $F_{41,213} = 2.607$, $p < 0.001$; Table S2.1; see also below). Post-hoc testing indicated lowest EEG delta/theta activity (4–5 Hz) during the first morning (CT −13 until CT −7; Fig. 3a). It then increased significantly during night time (starting at CT 5) and remained higher during the entire second day (i.e. all time points after CT 5) compared to the first morning. EEG delta/theta activity showed two statistically determined maxima, one in the morning (at CT 10) and one in the late afternoon (at CT 20; $p < 0.05$).

EEG alpha (10–14 Hz) and sigma/beta activity (15.5–23 Hz) were also at their lowest level at the beginning of the CR (CT −13 until CT −11; Fig. 3a). Compared to these low levels, EEG power density in both frequency ranges significantly increased (EEG alpha activity starting at CT −6; sigma/beta activity starting at CT 4). EEG alpha activity showed peaks which were statistically determined to occur during both evenings (at CT −4 and at CT 20) while EEG sigma/beta activity only showed one significant peak in the second evening (at CT 20). In between both peaks, EEG alpha activity significantly decreased with a trough at CT 3. Compared to CT 20 (i.e. directly prior to WMZ 2; see also below) EEG alpha as well as sigma/beta activity was significantly lower from CT 21 until CT 26 ($p < 0.05$).

Subjective and objective sleepiness changes during the wake maintenance zones. The effect of the WMZ on subjective and objective sleepiness was investigated via the same three analyses which were performed for cognitive performance tests (see Fig. S1). First we compared the absolute levels of sleepiness between the two WMZs (WMZ 1 vs. WMZ 2). Participants were significantly less sleepy during WMZ 1 compared to WMZ 2 ($F_{1,17} = 9.495$, $p = 0.007$, $d = 1.178$), and in the hour prior to WMZ 1, participants were also significantly less sleepy compared to the hour prior to WMZ 2 (PH 1 vs. PH 2; $F_{1,11} = 5.388$, $p = 0.041$, $d = 1.028$; Fig. 2h; Table S2.2.1). During WMZ 1, absolute EEG delta/theta activity (4. 0–5.0 Hz) was significantly lower than during WMZ 2 ($F_{1,27} = 21.460$, $p < 0.001$, $d = 1.177$), while absolute EEG alpha (10–14 Hz) and sigma/beta activity (15.5–23 Hz) showed no significant differences between the WMZs (Fig. 3a). In the hour prior to WMZ 1 compared to the hour prior to WMZ 2 (PH 1 vs. PH 2) there was also significantly lower EEG delta/theta ($F_{1,19} = 12.213$, $p = 0.002$, $d = 1.484$) and sigma/beta activity ($F_{1,11} = 8.063$, $p = 0.017$, $d = 1.097$) while EEG alpha activity showed no significant difference between the hours prior to the WMZs (Table S2.2.1).

We then compared subjective sleepiness and the waking EEG during each WMZ with the preceding hour (WMZ 1 vs. PH 1; and WMZ 2 vs. PH 2). Subjective sleepiness was not significantly reduced during either WMZ. In the wake EEG (0.5–25 Hz) we found significantly lower power density during WMZ 1 compared to the prior hour in the EEG delta/theta frequency range between 3.0–7.0 Hz ($F_{1,38} = 4.519$, $p = 0.040$, $d = -0.643$).

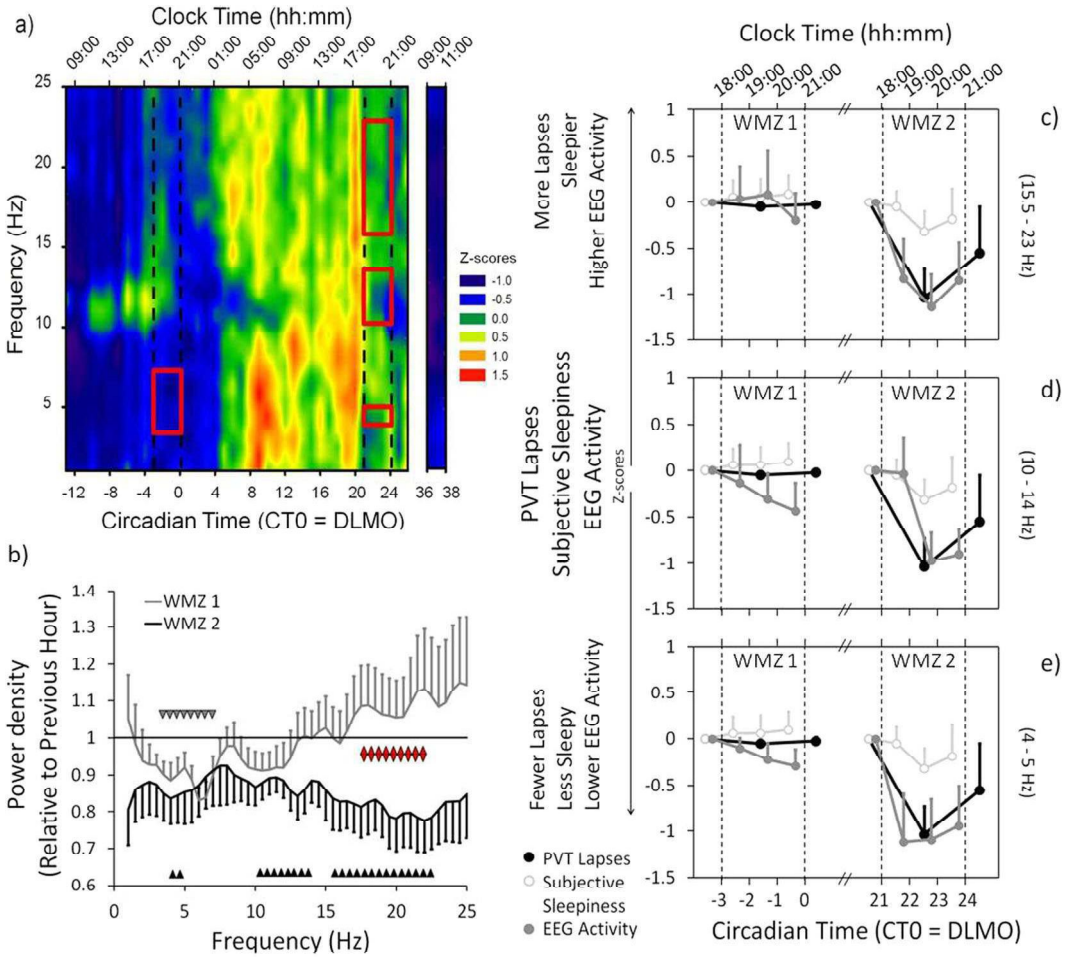

Figure 3. Wake-EEG, PVT lapses and subjective sleepiness during the WMZs. (**a**) Heat plot of the wake-EEG power density (standardized data; 0.5 to 25 Hz) over the 40 h CR and during the 2 h after the recovery night (frontal derivation; F4). Blue colors = lower EEG power density. Red colors = higher EEG power density. The upper x-axis shows the mean clock time (hh:mm). The lower x-axis shows the circadian time in hours relative to the DLMO of the first evening (=CT 0). The two sets of dotted lines indicate the time ranges of the two WMZs. The red rectangles show the frequency bins with significantly lower EEG power density during the WMZ compared to the preceding hour (WMZ 1: delta/theta range 3.0–7.0 Hz; WMZ 2: delta/theta 4.0–5.0 Hz, alpha 10.0–14.0 Hz and sigma/beta 15.5–23.0 Hz). (**b**) Changes in EEG power density during WMZ 1 (grey line) and WMZ 2 (black line) relative to the preceding hour. Frequency bins with significantly lower power density during WMZ 1 are indicated by grey downward triangles and during WMZ 2 by black upward triangles. Red triangles show the frequency bins (high sigma/beta range 17.5–22.5 Hz) with statistically significant differences between both WMZs (while expressed relative to the preceding hour). (**c**, **d** & **e**) All three panels show subjective sleepiness (open circles) and PVT lapses (closed black circles) during both WMZs relative to the preceding hour. Results for WMZ 1 are shown on the left and for WMZ 2 on the right side. Each panel compares the change in subjective sleepiness and PVT performance with one of the EEG frequency ranges (closed grey circles) which showed a reduction in EEG power density during WMZ 2 (from top to bottom: sigma/beta, alpha and delta/theta ranges). The upper x-axis shows the mean clock time. The lower x-axis shows the circadian time in hours relative to DLMO. The two sets of dotted lines indicate the time ranges of the WMZs. All data was standardized (z-scores). Mean ± SEM.

During WMZ 2 EEG power density was significantly lower in the delta/theta (4.0–5.0 Hz; $F_{1,38} = 11.901$, $p = 0.001$, $d = -1.021$), alpha (10.0–14.0 Hz; $F_{1,35} = 5.900$, $p = 0.020$, $d = -0.792$) and sigma/beta (15.5–23.0 Hz; $F_{1,31} = 7.612$, $p = 0.010$, $d = -0.805$) frequency ranges compared to the hour prior to WMZ 2 (Fig. 3a,b; Table S2.2.2).

As in the analysis of cognitive performance, we determined whether extended wakefulness had a differential impact on the change in subjective and objective sleepiness between both WMZs (WMZ 1/PH 1 vs. WMZ2/PH 2). The changes between both WMZs showed no difference in subjective sleepiness, but revealed a significantly stronger reduction in EEG power density during WMZ 2 in the high sigma/beta range (17.5–22.5 Hz) when compared to WMZ 1 ($F_{1,10} = 6.715$, $p = 0.027$; Fig. 3b; Table S2.2.3).

EEG power density reductions during the WMZ coincided with stable subjective sleepiness and improved cognitive performance. The finding that the PVT lapses showed a significantly greater decrease during WMZ 2 and the finding of a larger reduction in EEG sigma/beta activity during WMZ 2 compared to WMZ 1, warranted a closer look at the differences in the time courses between cognitive performance (as assessed in the PVT) and subjective/objective sleepiness during both WMZs. As visually apparent in Fig. 3c–e (the data is expressed relative to the hour preceding each WMZ), PVT lapses, subjective sleepiness (see also Fig. S2) and EEG beta activity remained constant during WMZ 1 whereas EEG delta/theta and alpha activity showed a significant reduction ($p < 0.05$). During WMZ 2, the EEG reductions were significantly more pronounced and also EEG sigma/beta activity showed a significant reduction while PVT lapses improved (EEG delta/theta activity: $F_{1,67} = 11.414$, $p = 0.001$, $d = -0.835$; alpha activity: $F_{1,68} = 2.075$, $p = 0.035$, $d = -0.335$; sigma/beta activity: $F_{1,68} = 11.679$, $p = 0.001$, $d = -0.794$; PVT lapses: $F_{1,39} = 7.766$, $p = 0.008$, $d = -1.210$; Main effects of WMZ; Table S2.3).

Subjective and objective sleepiness after the recovery night. After the recovery night and during polychromatic bright light exposure (see Methods) subjective sleepiness returned to levels that were no longer significantly different from the first morning of the CR (i.e. $p > 0.05$; Fig. 2h). Also, EEG power density (0.5–25 Hz) returned to the level of the start of the constant routine (Fig. 3a).

Associations between subjective and objective sleepiness, and cognitive performance. A correlation analysis showed that subjective sleepiness across the 40 h CR protocol was significantly associated with many of the cognitive performance tests. The sleepier participants rated themselves, the worse they performed on the PVT, the Go/No-Go test, the 2-Back Test, the Word-Memory Test, and Negative Affect Test (r ranged from −0.500 to 0.489 and $p < 0.032$; see Table S3.0 for all exact r and p-values). Subjective sleepiness did not show significant correlations with the Addition Task, the 3-Back Test or the Abstract Reasoning Task.

The EEG power density during the 40 h CR was also associated with subjective sleepiness and cognitive performance. In this analysis, the same frequency bands were used that were previously shown to be affected by the WMZs. An increase in EEG delta/theta activity (4–5 Hz) significantly correlated with greater subjective sleepiness and worse performance on most cognitive tests, except for the 3-Back and Addition Task (r ranged from −0.431 to 0.519; $p < 0.018$). An increase in EEG alpha activity (10–14 Hz) did not significantly correlate with subjective sleepiness but correlated significantly with worse performance on the PVT, the 2-Back Test and the Negative Affect Test (r ranged from −0.158 to 0.268; $p < 0.034$). Higher EEG sigma/beta activity (15.5–23 Hz) was significantly associated with an increase in subjective sleepiness and also a worsening of performance on the PVT, Go/No-Go, 2-Back, Word-Pair Memory Test, and the Negative Affect Test (r ranged from −0.322 to 0.328; $p < 0.024$).

Sleep stages during the baseline and the recovery night. Sleep during the baseline night was compared to sleep during the recovery night (see also Table S4). Participants had (as expected) significantly shorter sleep onset latency (SOL) and a significantly longer total sleep time (TST) in the recovery night (SOL: $F_{1,9} = 8.152$, $p = 0.018$; TST: $F_{1,9} = 5.070$, $p = 0.049$). During the recovery night participants had also significantly more deep sleep (stage N3; $F_{1,12} = 59.252$, $p < 0.001$) than during the baseline night. This occurred at the cost of significantly less light sleep (stage N1: $F_{1,12} = 20.894$, $p = 0.001$; and stage N2: $F_{1,10} = 19.271$, $p = 0.001$). Deep sleep latency was also significantly shorter, whereas wakefulness after sleep onset (WASO), sleep efficiency (SE), REM sleep latency and REM sleep showed no significant differences between baseline and recovery night (N3 onset latency: $F_{1,22} = 30.753$, $p < 0.001$; WASO: $F_{1,10} = 1.266$, $p = 0.286$; SE: $F_{1,10} = 1.010$, $p = 0.338$; REM sleep latency: $F_{1,12} = 0.802$, $p = 0.388$; REM sleep: $F_{1,11} = 0.441$, $p = 0.520$).

Discussion

During 40 hours of sleep deprivation in a CR protocol, performance of sustained attention (PVT) and response inhibition (Go/No-Go test) showed a circadian modulation with improvements during the WMZs, while performance on higher executive functioning tasks did not. Subjective sleepiness showed a similar time course as sustained attention, but was not significantly reduced during the WMZs. EEG delta/theta and sigma/beta activity increased over time but also demonstrated a circadian influence by revealing a significant power reduction during the WMZs when compared to the preceding hour. This reduction, together with a reduction in EEG alpha activity, was more pronounced in the second WMZ and coincided with stable subjective sleepiness and improved cognitive performance.

As expected and shown in previous studies[16,18] cognitive performance and sleepiness became worse between the first and the second evening which can be attributed to sleep deprivation effects. We found that the alerting effect during the WMZs was most evident during the second WMZ but this does not necessarily mean that the alerting effect of the first WMZ was much weaker. The hour preceding the WMZ was used as a baseline to compare the effects of the WMZs. In the hour preceding the first WMZ participants were still quite alert since it occurred during the late afternoon on a day when the participants were well rested. Therefore, the alerting effect during the first WMZ might seem smaller due to a ceiling effect.

We observed the clearest alerting effect of both WMZs in objective measures (EEG activity) and in the less difficult cognitive tasks. The absence of a WMZ effect on executive functioning could be explained by a lower susceptibility to sleep deprivation or mechanisms to compensate for greater sleepiness. A previous study found that negative effects of sleep deprivation on executive functioning could be compensated for by stronger activation of cerebral responses promoting task specific attention, or by activation of cerebral responses that were not active when the task was performed in well rested conditions[27]. Also, all our participants had to practice all the tests on the day preceding the 40 h constant routine in order to familiarize themselves with the cognitive tasks. Yet, any learning effects during the CR cannot fully be ruled out, even though we used different stimuli which were randomly presented for each task. We did not ask for the strategies subjects used to master the task. Therefore, it

might be that the strategy to complete a task may have changed during the CR and thus, task performance could be, at least in part, the result of changes in task strategy.

Most previous studies report improvements during the WMZ in sustained attention tests, and some also showed improvements in memory tests, as well as lower subjective sleepiness, while others found no changes during the WMZ[15–24]. We found improvements during the WMZs in sustained attention and response inhibition when we compared the WMZs to the preceding hour. These results are similar to a previous study, where improvements in the visual version of the PVT and the digit symbol substitution test were found during the WMZs on both evenings of a 50 h CR[16]. That same study also reported better performance for an auditory version of the PVT and for subjective sleepiness only during the WMZ of the second evening[16]. We also found an improvement in the auditory PVT only during the WMZ of the second evening, but no significant reduction of subjective sleepiness during both WMZs. Although in the evenings the performance decline in sustained attention as well as the augmentation of subjective sleepiness became steepest directly after DLMO (see Fig. S2). Thus, in our participants the circadian arousal signal during the WMZs may still be strong enough to 'counteract' homeostatic sleep pressure, since it kept subjective sleepiness stable until the 'sleep gate' (as the time around DLMO has been called[9]) was opened.

Low frequency EEG activity has been shown to correlate with worse cognitive performance and more lapses in sustained attention during extended wake episodes, which makes it a useful measure of objective sleepiness[3,18,28]. We also found that higher EEG delta/theta activity correlated with higher subjective sleepiness and a decline in most cognitive tests. In the EEG delta/theta range, which is known to be mainly under homeostatic control, we found a lower activity during the first WMZ and an increase over time leading to significant higher absolute EEG power during the second WMZ. Our results are in agreement with a model describing the time course of sleep propensity by a multiplicative interaction of two sleep drives, a homeostatic and a circadian one[29,30]. This model would correctly predict the presence of both WMZs, and in addition would predict the sleep propensity in WMZ 2 to be higher than in WMZ 1 if the circadian drive for sleep never attains zero values[29].

Our results showed a decrease of EEG activity in low (delta/theta) and higher (alpha, sigma/beta) frequency ranges during the WMZs when compared to the preceding hour. EEG power in these frequency ranges also demonstrated a significant modulation over time during the 40 h CR, as has been shown by others[31–33]. The potential influence of the circadian arousal signal specifically during the WMZ might be illustrated by the differential decline in EEG activity when we compared both WMZs. The reduction in the EEG sigma/beta frequency range was significantly stronger during the second compared to the first WMZ, suggesting that EEG power density changes in this frequency range are contributing to modulations of the circadian arousal signal, depending on prior duration of wakefulness.

We could confirm the circadian modulation of the time course of EEG alpha activity as shown by others[32]. The peak of EEG alpha activity on both days was in the afternoon just prior to the start of the WMZs. While subjective sleepiness remained stable and cognitive performance remained stable or was even improved during the WMZs, EEG alpha activity declined during the WMZ. This is interesting since the neurobehavioral substrate of the circadian modulation of alertness and cognitive performance is still not fully understood. A recent study by Muto and colleagues showed for the first time that functional MRI responses during sustained attention tests demonstrated a clear circadian modulation (especially in subcortical areas like the midbrain, cerebellum, basal ganglia, and thalamus)[34]. They also showed that this circadian modulation was closely related to the melatonin secretion pattern, with increased cortical responses during the WMZ immediately before the DLMO. Another study showed that during the WMZ, the postero-lateral hypothalamus is responsible for integration of the homeostatic sleep pressure and the circadian modulation of cognitive performance[25]. These studies are beginning to elucidate the underlying mechanisms of the brain that give rise to the circadian modulation of cognitive performance. Our findings of differential changes of EEG activity during the WMZs, depending on prior duration of wakefulness, may add to this understanding.

The reason why we found a WMZ effect in objective but not in subjective sleepiness could also be because of dissociation between the two, likely caused by a differential perception of subjective sleepiness. And it indicates a subjective adaptation to sleep depth as was shown in studies with chronic sleep restriction[35]. While many studies have, other studies have not, found correlations between subjective and objective sleepiness, and it has been hypothesized that the two reflect different physiological mechanisms underlying sleepiness[36–38]. We did find correlations in the time course over the 40 h CR between subjective sleepiness and delta/theta or sigma/beta EEG activity (see Table S3). The small effect size for subjective sleepiness indicates that our (small) sample size may have led to low statistical power for subjective measures, while the statistical power for objective sleepiness measures was large enough to reveal significant differences, as shown by the large effect sizes. Thus, the differences between subjective and objective sleepiness measures might also reflect the discrepancy in statistical power between the two variables, as shown by the different effect sizes.

We are aware that by analyzing the data in "three different ways" (see Results) we used parts of the same data for three different comparisons. But, since the comparisons of absolute and relative data are based on different a priori assumptions and were used in separate regression models, we refrained from using an a priori Bonferroni adjustment. Nevertheless, p-values within each model were adjusted for multiple comparisons by using the LSD method (see Methods).

We included only healthy young male participants. Perhaps we would have seen more of a WMZ alerting effect if we had included female participants since a recent study showed a stronger circadian rhythm in cognitive performance among women[23]. Including other age groups would also be important. One reason why we only included males was that the study was part of a larger project with genetic samples where the group was required to have as little hormonal and other variability as possible.

Our results may be of particular interest concerning shift work. Especially during night shifts, there is a misalignment between the sleep-wake cycle and the circadian rhythm of alertness and cognitive performance. Taking

the timing of the WMZ into account could be useful for example when timing naps. On the other hand, late chronotypes may benefit especially from the WMZ and experience fewer problems with night shifts if their WMZ overlaps with at least part of the night shift.

After the recovery night, performance on all cognitive tests returned to baseline levels (start of CR). In fact, the reaction times of the PVT were even faster than at the start of the CR when the participants were well rested. This contradicts previous findings that one 8 h recovery night is insufficient for complete cognitive recovery after acute sleep deprivation[39–42]. However, our setting was different because our study included a bright light exposure in the morning after the recovery night which may have aided the full cognitive recovery.

To summarize, we found that after 37.5 hours of extended wakefulness (WMZ 2) the circadian influence on the EEG delta/theta, alpha and sigma/beta ranges was stronger than under conditions of 'normal' sleep pressure (i.e. WMZ 1). Also in cognitive performance, the improvements during the second WMZ were more pronounced than during the first WMZ. The decrease in EEG activity during the WMZs occurred while subjective sleepiness remained stable and cognitive performance either remained stable or even improved. The differential changes in EEG power and cognitive performance during the WMZs reflect that the circadian arousal signal is modulated by the duration of prior wakefulness.

Methods

Participants. Participants were recruited via flyers at local universities. Twelve male participants were included in the study (age: 25.3 ± 2.6 yrs; mean ± SD). All participants completed a medical screening, an interview and filled out five screening questionnaires. To be included the participants had to be healthy and without any psychiatric or sleep disorders. They were only included if they were not taking any medications and if they were non-smokers. The five screening questionnaires were: a general entrance questionnaire, the Pittsburgh Sleep Questionnaire Index[43] (3.4 ± 0.9; mean ± SD), the Morningness-Eveningness Questionnaire[44] (50.3 ± 7.4; mean ± SD), the Munich Chronotype Questionnaire[45] (4.5 ± 0.6; mean ± SD) and the Seasonal Pattern Assessment Questionnaire[46] (7.1 ± 2.6; mean ± SD). Other inclusion criteria were no night shift work during the last eight weeks and no travel to other time zones in the last three months. The first night in the laboratory served as adaptation night and was polysomnographically recorded. None of the participants had periodic leg movements (PLM; score with arousals; cutoff < 10/h), or a sleep disorder (cutoff: apnoea/hypopnea index < 15/h). All participants gave a written informed consent and the study was approved by the local ethical committee of the Charité University Medicine Berlin (Germany) and conformed to the tenets of the Declaration of Helsinki.

Study design. Participants kept their regular habitual bedtimes for one week preceding the study (controlled by actigraphy and sleep diaries; habitual bedtime 23:50 ± 0:43; habitual wake time 7:51 ± 0:43; mean ± SD). On day 1 each participant came to the laboratory in the evening 3 h prior to habitual bedtime (Fig. 1). No more than one participant visited the laboratory at the same time. The first night was an adaptation night of approximately 8 h. On day 2 the participants spend the day in the laboratory with room lighting (LED ceiling lights; ≈500 lx in a vertical direction at eye level; 2800 K; 1.85 W/m^2). In the evening of day 2, six hours prior to habitual bedtime, the participants stayed in dim light (<5 lx) and hourly saliva samples were collected. The dim light was produced by a standing luminaire with a halogen bulb (polychromatic white light). The standing luminaire was adjusted to be lower than 5 lx (in a vertical direction anywhere in the room) as measured by a luxmeter (Showtec, Digital Luxmeter) and was kept at this illuminance level on the evening of day 2 and throughout the constant routine. The saliva samples were assayed for melatonin concentrations and the timing of the onset of melatonin concentrations in dim light (DLMO) was used as the circadian phase marker. This was followed by a baseline night (8 h) after which the 40 h CR protocol started. During the CR participants remained in dim light (<5 lx), stayed in bed in a semi-recumbent position (~45°) at all times, and received iso-caloric snack opportunities every hour (150 kcal). The laptop screen used during the cognitive tests was maximally dimmed so that illuminance measured at eye level in a vertical angle of gaze was below 5 lx at all times. The participants had no information about the time of day and the use of cell-phones or tablets was not allowed. Saliva samples were collected hourly (see supplement), core body temperature (rectal probe; data not reported here) and the electroencephalogram were recorded continuously. The 40 h CR protocol was followed by an 8 h recovery night. On day 5, one hour after wake-up time participants were exposed to 3 h of polychromatic bright white light (1.300 lx; measured at eye level in a vertical angle of gaze) for purposes not reported here.

Cognitive tests and subjective sleepiness. Two cognitive test batteries with a total of 7 different tasks were performed during the CR alternating every two hours (on a laptop with dimmed screen). The first battery consisted of the N-Back Tests (with three levels: 0-Back, 2-Back, 3-Back Test)[47], a 5 min version of the auditory Psychomotor Vigilance Task (PVT)[48], a 3 min visual Addition Task[49], and a task where participants had to recognize negative emotions (Negative Affect)[50]. The second cognitive test battery consisted of a visual 3 min Go/No-Go task[51], a delayed recall Word-Pair Memory Task[52] and an Abstract Reasoning Task[53]. The Karolinska Sleepiness Scale (KSS)[54] was included in both batteries and was performed every hour at the beginning of the cognitive testing. For visual illustration, results were averaged every 1–2 hours for the entire study protocol and the morning following recovery sleep.

Wake-EEG and sleep recordings. Every hour, following the cognitive tests, participants performed the Karolinska Drowsiness Test (KDT; 3 min)[31]. They had to keep their eyes open, and refrain from moving, as well as blink as few times as possible. The recordings were performed with six EEG derivations (F3, F4, C3, C4, O1 and O2), referenced against mastoids (A1 and A2) using a Rembrandt system (Monet 24-CPU hardware, TMS International, Enschede, The Netherlands; and Rembrandt 7.5 software, Medcare Automation, Amsterdam, The Netherlands). The sampling rate of the EEG was 160 Hz and recordings were low-pass filtered (70 Hz) and

high-pass filtered (0.3 Hz). After study completion, the EEG MATLAB toolbox (The MathWorks, Inc., Natick, Massachusetts, United States) was used for manual artifact removal (movements and blinking) and spectral analysis. Spectral analysis was performed by applying a Fast Fourier Transformation in the range between 0.5 to 80.5 Hz with a resolution of 0.5 Hz. Here, we report data in the frequency range between 0.5 and 25 Hz from a frontal derivation (F4). Recordings from two out of the twelve subjects could not be included in the wake-EEG analysis due to technical problems.

Polysomnographic sleep recordings of the baseline and recovery night were visually scored in compliance with the Guidelines of the American Academy of Sleep Medicine (2007)[55]. The recovery night of one participant was excluded from the analysis due to technical problems during the recording.

Dim light melatonin onset. Dim light melatonin onset (DLMO) was used in order to assess individual circadian phase, determined by the software tool of Danilenko *et al.*[56]. The DLMO was defined as the time when the melatonin concentration exceeded two standard deviations (2 SD) after three low daytime time points[57]. See supplemental text for inter- and intra-assay values (page 17).

Timing of the wake maintenance zone. The WMZ was defined as the time range 3 h prior to DLMO on the first evening of the CR (i.e. DLMO = CT 0 which was on average at 21:17 ± 1:09 h; SD; range = 19:13 until 22:36). WMZ 1 was on the first day between 10.5 and 13.5 h after wake time and WMZ 2 on the second day between 34.5 and 37.5 h being awake (±0.9 h; SD).

Statistical analysis. All statistics were performed in IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp., Armonk, N.Y., USA). Cognitive performance, subjective sleepiness and EEG power densities were first expressed relative to the DLMO of the first CR evening (=CT 0). For cognitive performance, mixed linear models with fixed factor "TIME" and random factor "PARTICIPANT" were performed on $\log_{10}$-transformed data to analyze differences across the 40 h CR and to compare cognitive performance after the recovery night with the start of the CR. In a second step, the effects of the WMZ were compared to the preceding hour by mixed linear models with the fixed factors "TIME POINT" (which was either "during WMZ" or "hour prior to WMZ") and "DAY" (1st biological day or 2nd biological day) and again the random factor "PARTICIPANT". The same mixed linear models were performed on the $\log_{10}$-transformed EEG power densities of the KDTs in the wake-EEG for every 0.5 Hz frequency bin in the range from 0.5 to 25 Hz. In the analysis of the decline in EEG frequency ranges during the WMZs, mixed linear models were performed with the fixed factors "TIME" (= time points around the WMZs) and "WMZ" (WMZ 1 or WMZ 2). All post-hoc tests were corrected for multiple comparisons by the Least Significance Test (LSD). Correlation analysis was performed by using a Spearman's Rho correlation for hourly bins of cognitive performance, subjective sleepiness and EEG power bins in three different frequency ranges.

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Author Contributions

M.M. and D.K. designed the experiment; J.D.Z., S.W., A.P., F.B., A.W., M.Z. and M.M. performed the study; M.M., J.D.Z., S.W., A.P., M.Z. analyzed the data; J.D.Z. prepared the Figures; J.D.Z., M.M., F.B. wrote the manuscript; all authors edited and reviewed the manuscript and all authors approved the final version of the manuscript.

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Supplemental Material

The alerting effect of the wake maintenance zone during 40 hours of sleep deprivation

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Cognitive Tests	Main Effect of TIME
PVT Median Reaction Times	$F_{1,20} = 4.042$, $p < 0.001$
PVT Lapses	$F_{1,20} = 3.208$, $p < 0.001$
Go/No-Go	$F_{1,20} = 4.815$, $p < 0.001$
2-Back	$F_{1,20} = 3.519$, $p < 0.001$
3-Back	ns
Word-Memory	ns
Addition Task	$F_{1,20} = 3.611$, $p < 0.001$
Abstract Reasoning	ns
Negative Affect	$F_{1,20} = 2.810$; $p < 0.001$

Table S1.1. Cognitive performance across 40 hours. The first column shows: PVT median reaction times in milliseconds; number of PVT lapses; % correct on the Go/No-Go; % correct on the 2-Back test; % correct on the 3-Back Test; % correct on the Word-Memory Test; Efficiency on the Addition Task (% correct / Reaction Time); % correct on the Abstract Reasoning Test; % correct on Negative Affect. The second column shows the F- and p-values for the statistical variation of the time course of cognitive performance tests across 40 h of extended wakefulness (= main effect of TIME); ns = not significant.

Cognitive Tests	WMZ 1 vs. WMZ 2				Hour before WMZ 1 vs. Hour before WMZ 2			
	F- and p-values	Effect Size (d)	WMZ 1 Mean (± SD)	WMZ 2 Mean (± SD)	F- and p-values	Effect Size (d)	Hour before WMZ 1 Mean (± SD)	Hour before WMZ 2 Mean (± SD)
PVT Median Reaction Times	ns	0.293	271.3 (24.2)	279.0 (28.4)	ns	0.329	270.9 (20.5)	279.1 (29.0)
PVT Lapses	p = 0.047	0.968	0.3 (0.7)	2.2 (2.7)	p = 0.008	1.108	0.5 (0.9)	5.5 (6.3)
Go/No-Go	$F_{1,11}$ = 7.084 p = 0.022	-0.747	94.5 (4.9)	87.9 (11.5)	$F_{1,10}$ = 6.774 p = 0.027	-0.697	92.3 (6.6)	85.1 (12.9)
2-Back	$F_{1,16}$ = 13.921 p = 0.002	-1.160	97.7 (2.3)	92.4 (6.0)	ns	-0.623	95.5 (5.2)	91.8 (6.4)
3-Back	ns	-0.501	87.0 (5.8)	82.4 (11.6)	$F_{1,13}$ = 8.180 p = 0.014	-0.471	85.2 (10.2)	79.7 (12.9)
Word-Memory	ns	-0.094	90.0 (16.5)	88.3 (18.9)	$F_{1,10}$ = 10.495 p = 0.021	-0.804	95.0 (8.0)	85.0 (5.7)
Addition Task	ns	-0.185	0.020 (0.007)	0.019 (0.008)	$F_{1,11}$ = 10.729 p = 0.007	-1.135	0.023 (0.004)	0.017 (0.006)
Abstract Reasoning	ns	-0.263	58.1 (27.8)	51.9 (18.6)	ns	-0.336	61.4 (21.6)	51.9 (18.0)
Negative Affect	ns	-0.646	74.2 (12.4)	66.2 (12.4)	ns	-0.885	74.3 (9.9)	66.0 (8.9)

Table S1.2.1. Cognitive performance: comparison between WMZs and between prior hours. The first column of the table lists the cognitive performance tests. The second column shows the F- and p-values of the comparison between WMZ 1 and WMZ 2. The third column shows the effect size (Cohen's d). The fourth and fifth columns show the means (± SD). The sixth column shows the F- and p-values of the comparison between the hour before WMZ 1 and the hour before WMZ 2. The seventh column shows the effect size (Cohen's d). The eighth and ninth columns show the means (± SD). Since PVT lapses were not normally distributed they were analyzed with the Wilcoxon Test. The cognitive performance tests are presented as: PVT median reaction times in milliseconds; number of PVT lapses; % correct on the Go/No-Go; % correct on the 2-Back test; % correct on the 3-Back test; % correct on the Word-Memory test; Efficiency on the Addition Task (% correct / reaction time); % correct on the Abstract Reasoning Test; % correct on Negative Affect; ns = not significant.

Cognitive Tests	Preceding Hour vs. WMZ 1				Preceding Hour vs. WMZ 2			
	F- and p-values	Effect Size (d)	Hour prior to WMZ 1 Mean (± SD)	WMZ 1 Mean (± SD)	F- and p-values	Effect Size (d)	Hour prior to WMZ 2 Mean (± SD)	WMZ 2 Mean (± SD)
PVT Median Reaction Times	ns	0.018	270.9 (20.5)	271.3 (24.2)	ns	-0.004	279.1 (29.0)	279.0 (28.4)
PVT Lapses	ns	-0.289	0.5 (0.9)	0.3 (0.7)	p = 0.012	-0.687	5.5 (6.3)	2.2 (2.7)
Go/No-Go	$F_{1,12} = 5.142$ p = 0.043	0.380	92.3 (6.6)	94.5 (4.9)	ns	0.227	85.1 (12.9)	87.9 (11.5)
2-Back	ns	0.550	95.5 (5.2)	97.7 (2.3)	ns	0.098	91.8 (6.4)	92.4 (6.0)
3-Back	ns	0.224	85.2 (10.2)	87.0 (5.8)	ns	0.223	79.7 (12.9)	82.4 (11.6)
Word-Memory	ns	-0.386	95.0 (8.0)	90.0 (16.5)	ns	0.191	85.0 (5.7)	88.3 (18.9)
Addition Task	ns	-0.501	0.023 (0.004)	0.020 (0.007)	ns	0.253	0.017 (0.006)	0.019 (0.008)
Abstract Reasoning	ns	-0.131	61.4 (21.6)	58.1 (27.8)	ns	-0.154	51.9 (18.0)	51.9 (18.6)
Negative Affect	ns	0.013	74.3 (9.9)	74.2 (12.4)	ns	0.015	66.0 (8.9)	66.2 (12.4)

Table S1.2.2. Cognitive performance: comparisons of each WMZ with its preceding hour. The first column of the table lists the cognitive performance tests. The second column shows the F- and p-values of the comparison between WMZ 1 and its preceding hour. The third column shows the effect size (Cohen's d; see also Fig. S3). The fourth and fifth columns show the means (± SD). The sixth column shows the F- and p- values of the comparison between WMZ 2 and its preceding hour. The seventh column shows the effect size (Cohen's d). The eighth and ninth columns show the means (± SD). Since PVT lapses were not normally distributed they were analyzed with the Wilcoxon Test. The cognitive performance tests are presented as: PVT median reaction times in ms; number of PVT lapses; % correct on the Go/No-Go; % correct on the 2-Back test; % correct on the 3-Back test; % correct on the Word-Memory test; Efficiency on the Addition Task (% correct / reaction time); % correct on the Abstract Reasoning Test; % correct on Negative Affect; ns = not significant.

Cognitive Tests	Change in WMZ 1 vs. Change in WMZ 2			
	F- and p-values	Effect Size (d)	WMZ 1 Mean (± SD)	WMZ 2 Mean (± SD)
PVT Median Reaction Times	ns	0.057	1.0 (0.04)	1.0 (0.04)
PVT Lapses	p = 0.009	-0.986	-0.3 (1.1)	-3.3 (4.3)
Go/No-Go	ns	0.207	1.03 (0.05)	1.04 (0.08)
2-Back	ns	-0.119	1.02 (0.06)	1.01 (0.06)
3-Back	ns	0.228	1.02 (0.11)	1.05 (0.16)
Word-Memory	ns	0.491	0.95 (0.17)	1.06 (0.26)
Addition Task	$F_{1,11} = 6.843$ p = 0.025	0.808	0.9 (0.3)	1.1 (0.2)
Abstract Reasoning	ns	0.047	1.06 (0.72)	1.09 (0.48)
Negative Affect	ns	-0.012	1.01 (0.16)	1.01 (0.19)

Table S1.2.3. Cognitive performance: comparisons of changes between wake maintenance zones. The first column of the table lists the cognitive performance tests. The second column shows the F- and p-values for the comparison of the changes during WMZ 1 and WMZ 2. The third column shows the effect size (Cohen's d). The fourth and fifth columns show the means (± SD). For PVT lapses the Wilcoxon test was performed. The cognitive performance tests are presented as relative values (each WMZ relative to its preceding hour); ns = not significant.

Subjective Sleepiness/EEG Activity	Main Effect of TIME
Subjective Sleepiness	$F_{1,41} = 8.596$, $p < 0.001$
EEG delta/theta activity (4 - 5 Hz)	$F_{41,232} = 6.812$, $p < 0.001$
EEG alpha activity (10 - 14 Hz)	$F_{41,208} = 2.861$, $p < 0.001$
EEG sigma/beta activity (15.5 - 23 Hz)	$F_{41,213} = 2.607$, $p < 0.001$

Table S2.1. Subjective and objective sleepiness across 40 hours. F- and p-values for the time course of subjective sleepiness and EEG frequency ranges across 40 h of extended wakefulness (= main effect of TIME).

Subjective Sleepiness/EEG Activity	WMZ 1 vs. WMZ 2		Hour before WMZ 1 vs. Hour before WMZ 2	
	F- and p-values	Effect Size (d)	F- and p-values	Effect Size (d)
Subjective Sleepiness	$F_{1,17} = 9.495$ $p = 0.007$	1.178	$F_{1,11} = 5.388$ $p = 0.041$	1.028
EEG delta/theta activity (4 - 5 Hz)	$F_{1,27} = 21.460$ $p < 0.001$	1.177	$F_{1,19} = 12.213$ $p = 0.002$	1.484
EEG alpha activity (10 - 14 Hz)	ns	0.242	ns	0.744
EEG sigma/beta activity (15.5 - 23 Hz)	ns	0.373	$F_{1,11} = 8.063$ $p = 0.017$	1.097

Table S2.2.1. Subjective and objective sleepiness: comparison between WMZs and between prior hours. The first column of the table lists the subjective sleepiness and EEG activity ranges. The second column shows the F- and p-values for the comparison between WMZ 1 and WMZ 2 with the effect size in the third column (Cohen's d). The fourth column shows the F- and p-values of the comparison between the hour prior to WMZ 1 and the hour prior to WMZ 2, with the effect size in the fifth column (Cohen's d); ns = not significant.

Subjective Sleepiness/EEG Activity	Preceding Hour vs. WMZ 1		Preceding Hour vs. WMZ 2	
	F- and p-values	Effect Size (d)	F- and p-values	Effect Size (d)
Subjective Sleepiness	ns	0.045	ns	-0.123
EEG delta/theta activity (3 - 7 Hz)	$F_{1,37} = 8.762$, $p = 0.005$	-0.850	ns	-1.037
EEG delta activity (4 - 5 Hz)	$F_{1,38} = 4.519$, $p = 0.040$	-0.643	$F_{1,38} = 11.901$, $p = 0.001$	-1.021
EEG alpha activity (10 - 14 Hz)	ns	-0.443	$F_{1,35} = 5.900$, $p = 0.020$	-0.792
EEG sigma/beta activity (15.5 - 23 Hz)	ns	-0.058	$F_{1,31} = 7.612$, $p = 0.010$	-0.805

Table S2.2.2. Subjective and objective sleepiness: comparisons of each WMZ with its preceding hour. The first column of the table lists the subjective sleepiness and EEG activity ranges. The second column shows the F- and p-values for the comparison between WMZ 1 and the preceding hour, with the effect size in the third column (Cohen's d). The fourth column shows the F- and p-values of the comparison between WMZ 2 and the preceding hour, with the effect size in the fifth column (Cohen's d); ns = not significant.

Subjective Sleepiness/EEG Activity	Change in WMZ 1 vs. Change in WMZ 2
Subjective Sleepiness	ns
EEG sigma/beta activity (17.5 - 22.5 Hz)	$F_{1,10} = 6.715$, $p = 0.027$

Table S2.2.3. Subjective and objective sleepiness: comparisons of changes between wake maintenance zones. The first column of the table lists the subjective sleepiness and EEG activity ranges. The second column shows the F- and p-values for the comparison of the changes during WMZ 1 and WMZ 2; ns = not significant.

	Main Effect of WMZ	Main Effect of TIME
PVT Lapses	$F_{1,39}$ = 7.766, p = 0.008	$F_{1,39}$ = 10.134, p = 0.003
Subjective Sleepiness	ns	ns
EEG delta/theta activity (4 - 5 Hz)	$F_{1,67}$ = 11.414, p = 0.001	$F_{3,63}$ = 2.865, p = 0.044
EEG alpha activity (10 - 14 Hz)	$F_{1,68}$ = 2.075, p = 0.035	$F_{3,65}$ = 4.635, p = 0.005
EEG sigma/beta activity (15.5 - 23 Hz)	$F_{1,68}$ = 11.679, p = 0.001	$F_{3,66}$ = 1.571, p = 0.034

Table S2.3. EEG power density reductions during the WMZ coincided with stable subjective sleepiness and improved cognitive performance. The first column lists the cognitive performance, subjective sleepiness and EEG activity ranges. The second column shows whether the magnitude of the decline during the WMZs was different between WMZ 1 compared to WMZ 2 (main effect of WMZ). The third column shows whether there was a significant decline over time during the WMZ (compared to the start of the WMZ; main effect of TIME). Only PVT lapses showed a significant interaction ($F_{1,39}$ = 7.766, p = 0.008); ns = not significant.

Subjective Sleepiness vs.:	**r**	**p-values**
PVT Median Reaction Times	r = 0.430	p < 0.001
PVT Lapses	r = 0.479	p < 0.001
Go/No-Go	r = -0.500	p < 0.001
2-Back	r = -0.250	p < 0.001
3-Back	r = -0.106	ns
Word-Memory	r = -0.259	p < 0.001
Addition Task	r = 0.002	ns
Abstract Reasoning	r = -0.121	ns
Negative Affect	r = -0.142	p = 0.031
Delta/Theta Activity (4 - 5 Hz) vs.:	**r**	**p-values**
Subjective Sleepiness	r = 0.506	p < 0.001
PVT Median Reaction Times	r = 0.455	p < 0.001
PVT Lapses	r = 0.519	p < 0.001
Go/No-Go	r = -0.431	p < 0.001
2-Back	r = -0.304	p < 0.001
3-Back	r = -0.143	ns
Word-Memory	r = -0.277	p < 0.001
Addition Task	r = -0.044	ns
Abstract Reasoning	r = -0.175	p = 0.017
Negative Affect	r = -0.309	p < 0.001
Alpha Activity (10 - 14 Hz) vs.:	**r**	**p-values**
Subjective Sleepiness	r = 0.060	ns
PVT Median Reaction Times	r = 0.167	p = 0.023
PVT Lapses	r = 0.232	p = 0.002
Go/No-Go	r = 0.015	ns
2-Back	r = 0.268	p = 0.001
3-Back	r = -0.057	ns
Word-Memory	r = -0.128	ns
Addition Task	r = -0.053	ns
Abstract Reasoning	r = 0.052	ns
Negative Affect	r = -0.158	p = 0.033
Sigma/Beta Activity (15.5 - 23 Hz) vs.:	**r**	**p-values**
Subjective Sleepiness	r = 0.257	p < 0.001
PVT Median Reaction Times	r = 0.328	p < 0.001
PVT Lapses	r = 0.326	p < 0.001
Go/No-Go	r = -0.205	p = 0.005
2-Back	r = -0.184	p = 0.018
3-Back	r = -0.060	ns
Word-Memory	r = -0.322	p < 0.001
Addition Task	r = 0.006	ns
Abstract Reasoning	r = -0.055	ns
Negative Affect	r = -0.167	p = 0.023

Table S3.0. Associations between subjective and objective sleepiness, and cognitive performance. A correlation analysis was performed on the time course over the 40 h CR. The r and p-values of the correlations between subjective sleepiness and cognitive performance as well as the correlations of EEG frequency ranges with subjective sleepiness and cognitive performance are shown. Correlations were performed with Spearman´s Rho since the data was not normally distributed; ns = not significant.

	Baseline Night Mean (± SD)	**Recovery Night** Mean (± SD)	**F- and p-values**
Time in Bed (TIB; min)	478.3 (9.1)	481.9 (4.2)	ns
Total Sleep Time (TST; min)	441.5 (42.6)	465.1 (17.8)	$F_{1,9} = 5.070$, $p = 0.049$
Sleep Onset Latency (SOL; min)	11.0 (9.6)	4.9 (2.5)	$F_{1,9} = 8.152$, $p = 0.018$
REM Sleep Onset Latency (min)	71.7 (30.6)	81.3 (34.6)	ns
N3 Onset Latency (min)	8.9 (3.6)	2.8 (1.9)	$F_{1,22} = 30.753$, $p < 0.001$
Stage N1 (min)	35.8 (10.0)	22.2 (9.3)	$F_{1,12} = 20.894$, $p = 0.001$
Stage N2 (min)	192.2 (27.9)	174.4 (22.1)	$F_{1,10} = 19.271$, $p = 0.001$
Stage N3 (min)	113.2 (29.8)	167.7 (33.3)	$F_{1,12} = 59.252$, $p < 0.001$
REM Sleep (min)	100.4 (28.4)	100.8 (22.5)	ns
NREM (S2+S3)	305.4 (31.6)	342.1 (24.6)	$F_{1,11} = 13.191$, $p = 0.004$
WASO (min)	25.6 (35.8)	12.3 (16.2)	ns
Sleep Efficiency (SE; %)	92.3 (8.7)	96.5 (3.7)	ns

Table S4. Polysomnographic sleep recordings. The first column lists the sleep variables with duration in minutes or %. The second column shows the values for the baseline night; mean (± SD). The third column shows the values for the recovery night; mean (± SD). The fourth column shows the significant F- and p-values. Abbreviations: Time in bed (TIB); Total sleep time (TST, sum of stages N1-3 and REM sleep); Sleep onset latency (SOL); REM sleep onset latency; N3 (deep sleep) onset latency; Stage N1; Stage N2; Stage N3; rapid eye movement (REM) sleep; non-REM (NREM) sleep; WASO = wake after sleep onset; Sleep efficiency (SE; TST / TIB *100).

Supplemental Figures

1. PH 1 ⟷ PH 2
   WMZ 1 ⟷ WMZ 2
2. WMZ 1 ⟷ PH 1
   WMZ 2 ⟷ PH 2
3. $\frac{\text{WMZ 1}}{\text{PH 1}} \longleftrightarrow \frac{\text{WMZ 2}}{\text{PH 2}}$

Figure S1. Statistical comparisons of the Wake Maintenance Zone. This figure gives an overview of the three ways in which the effects of the WMZ on cognitive performance and EEG activity were analyzed. (1.) First, we compared the absolute values between both WMZs and between the previous hours (PH). (2.) Secondly, each WMZ was compared to its respective previous hour. (3.) Finally, each WMZ was expressed relative to its previous hour after which the changes were compared between the two WMZs.

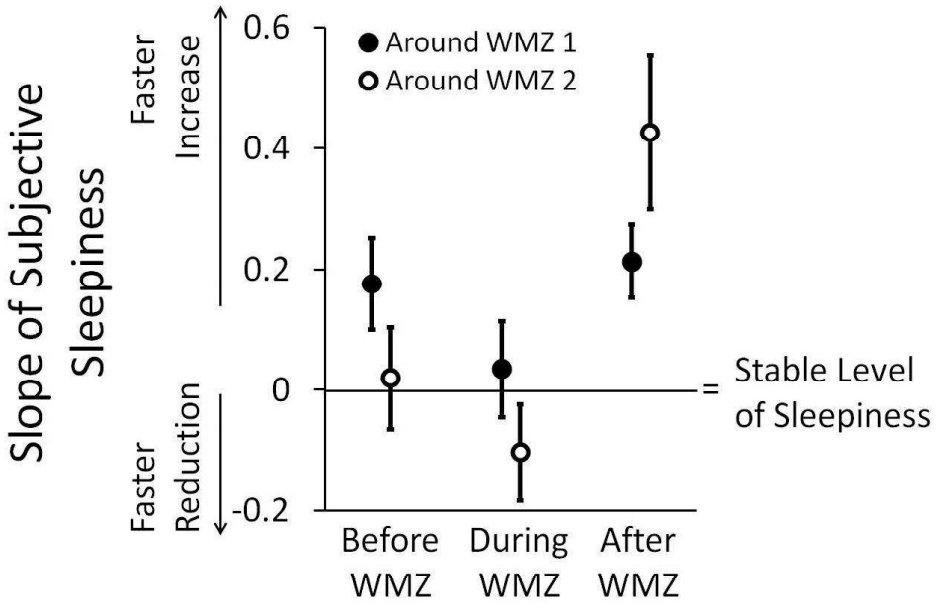

Figure S2. Slope of Subjective Sleepiness. Depicted are the slopes of the time course of subjective sleepiness during the 3 h before each WMZ (WMZ 1: CT -6 until CT -3; WMZ 2: CT 18 until CT 21), during the 3 h of each WMZ (WMZ 1: CT -3 until CT 0; WMZ 2: CT 21 until CT 24), and after each WMZ (WMZ 1: CT 0 until CT 2; WMZ 2: CT 24 until end of CR). The y-axis shows the magnitude of the slope of subjective sleepiness on z-transformed values (i.e. the speed of the increase/reduction of sleepiness). A positive slope indicates an increase of sleepiness and a negative slope indicates a reduction of sleepiness. A mixed model analysis showed a significant main effect for a difference between the three time windows ($F_{2,36} = 8.550$, $p = 0.001$) with a significantly steeper slope (i.e. greater increase of sleepiness) after the WMZs compared to the time before and during both WMZs. There was also a significant interaction between 'time window' and 'biological day' ($F_{2,36} = 3.497$, $p = 0.041$) with the post hoc showing a trend for a larger slope after WMZ 2 than after WMZ 1 ($p = 0.066$).

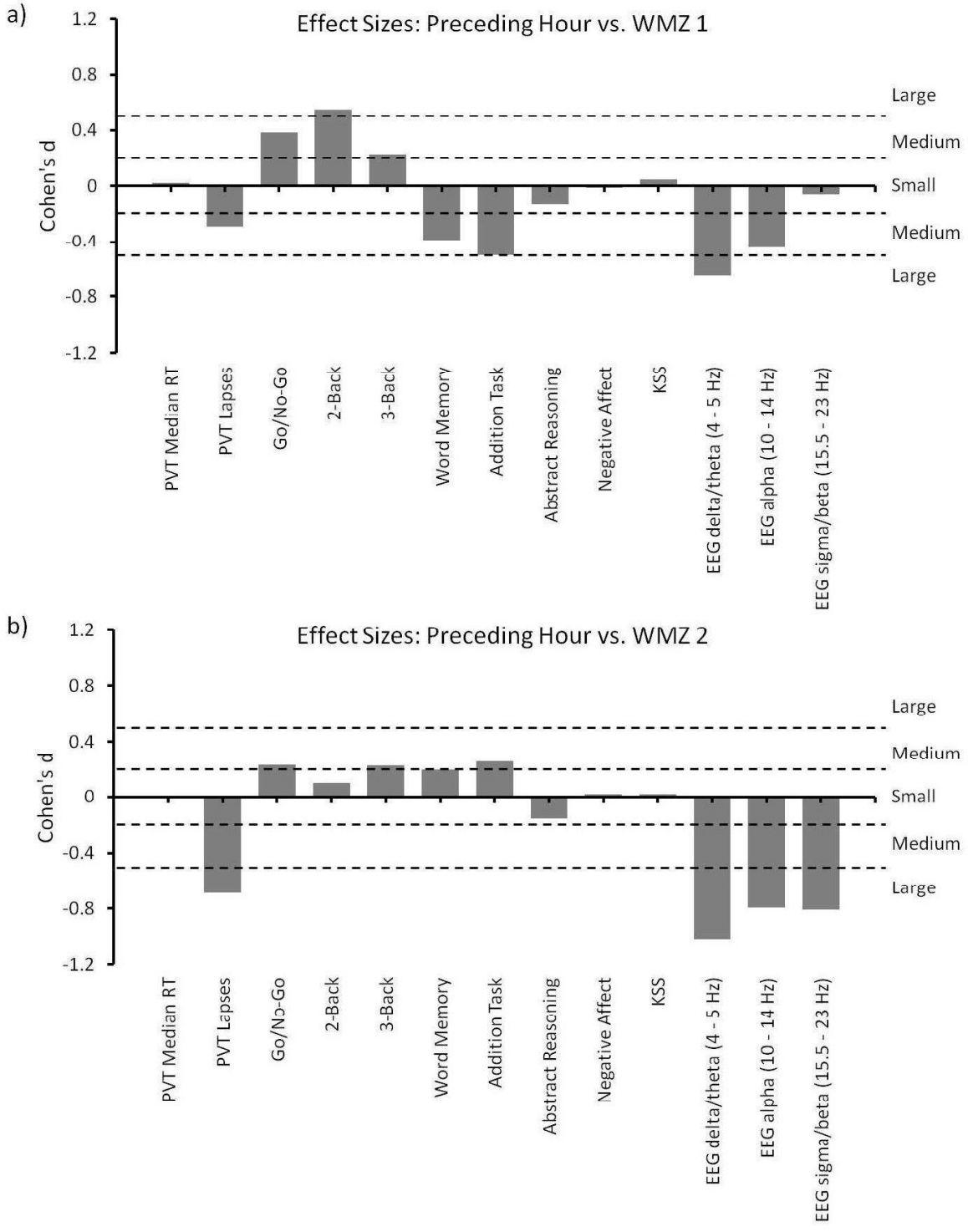

Figure S3. Effect Sizes. Depicted are the effect sizes (Cohen's d) for the comparison of (**a**) WMZ 1 versus its preceding hour and (**b**) WMZ 2 versus its preceding hour. Shown from left to right are: PVT median reaction times in ms; number of PVT lapses; % correct on the Go/No-Go;

% correct on the 2-Back Test; % correct on the 3-Back Test; % correct on the Word-Memory Test; Efficiency on the Addition Task (% correct / reaction time); % correct on the Abstract Reasoning Test; % correct on Negative Affect; subjective sleepiness (KSS); EEG delta/theta activity (4 – 5 Hz); EEG alpha activity (10 – 14 Hz); EEG sigma/beta activity (15.5 – 23 Hz). The dotted lines indicate the cut-offs for the effect size (small < 0.2; medium < 0.5; large > 0.5). A negative effect size indicates a reduction during the WMZ compared to its preceding hour.

Saliva samples

For salivary samples salivettes were used (Salivetten®; Sarstedt Ag & Co.; Nümbrecht; Germany). From these hourly saliva samples (starting 10 min after habitual wake time at the beginning of the CR) the concentration of melatonin was determined with radio-immuno assays by an external laboratory (IBL International GmbH, Hamburg; Germany). The intra-assay coefficients of variability for low- and high-dose control concentration probes were 10.3% and 6.4% (low range: 2.3 – 25.7%; high range: 0.6 – 16.1%). The inter-assay coefficients of variability for low- and high-dose control concentration probes were 12.3 pg/mL and 12.2 pg/mL.

Study 3: Daytime Lighting Study

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Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

Complete list of publications

(Journal impact factors according to Clarivate Analytics, 2018)

1 Regente J*, **de Zeeuw J***, Bes F, Nowozin C, Appelhoff S, Wahnschaffe A, Münch M[#], Kunz D[#]. Can short-wavelength depleted bright light during single simulated night shifts prevent circadian phase shifts? *Journal of Applied Ergonomics*. *Volume 61*, **2017**, p. 22-30 (* = co-first authors; [#] = co-last authors)
Journal impact factor (2018): 2.610

2 Münch M, Nowozin C, Regente J, Bes F, **de Zeeuw J**, Hädel S, Wahnschaffe A, Kunz D. Blue-enriched morning light as a countermeasure to light at the wrong time: effects on cognition, sleepiness, sleep, and circadian phase. *Neuropsychobiology*. *Volume 74*, **2017**, p. 207-218
Journal impact factor (2018): 1.675

3 Nowozin C, Wahnschaffe A, Rodenbeck A, **de Zeeuw J**, Hädel S, Kozakov R, Schöpp H, Münch M[#], Kunz D[#]. Applying melanopic lux to measure biological light effects on melatonin suppression and subjective sleepiness. *Current Alzheimer Research*. *Special Issue*, *Volume 14*, **2017**, p. 11 ([#] = co-last authors)
Journal impact factor (2018): 3.211

4 Wittenbrink N*, Ananthasubramaniam B*, Münch M*, Koller B, Maier B, Weschke C, Bes F, **de Zeeuw J**, Nowozin C, Wahnschaffe A, Wisniewski S, Zaleska M, Bartok O, Ashwal-Fluss R, Lammert H, Herzel H, Hummel M, Kadener S, Kunz D, Kramer A. High-accuracy determination of internal circadian time from a single blood sample. *Journal of Clinical Investigation*. *Volume 128*, **2018**, p. 3826-3839 (* = co-first authors)
Journal impact factor (2018): 12.282

5 **de Zeeuw J**, Wisniewski S, Papakonstantinou A, Bes F, Wahnschaffe A, Zaleska M, Kunz D[#], Münch M[#]. The alerting effect of the wake maintenance zone during 40 hours of sleep deprivation. *Scientific Reports*. *Volume 8*, **2018**, nr. 11012 ([#] = co-last authors)
Journal impact factor (2018): 4.011

6 **de Zeeuw J**, Papakonstantinou A, Nowozin C, Stotz S, Zaleska M, Hädel S, Bes F, Münch M[#], Kunz D[#]. Living in biological darkness: Objective sleepiness and the pupillary light responses are affected by different metameric lighting conditions during daytime. *Journal of Biological Rhythms*. *Volume 34*, **2019**, p. 410-431 ([#] = co-last authors)
Journal impact factor (2018): 2.473

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