Sleep alterations in the course of aging environmental inputs

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Chapter 4

Sleep and dim-light-at-night duration periods

Sleep network deterioration as a function of dim-light-at-night exposure duration in a mouse model

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To be submitted

Abstract

Artificial light has a widespread use, offering several positive effects on society. However, recent research associates nocturnal light exposure with deterioration of health and well-being. One possible aspect of these disadvantageous effects includes altered circadian timing and sleep disturbances. In the present study, C57BL/6J mice were exposed to either a 12:12 h light:dark cycle (75lux:0lux) or a 12:12h light:dim-light-at-night cycle (DLAN, 75lux:5lux) and sleep electroencephalogram (EEG) and the electromyogram were recorded after one day, one week, one month, and three months of exposure. In addition to baseline 24-h recordings, a 6-h sleep deprivation was performed. We found a gradual delay of the daily 24-h vigilance state rhythms with increasing DLAN exposure duration. Although sleep was already affected following the first day of DLAN exposure, and the response to sleep deprivation was reduced in all DLAN duration periods, the most severe effects on sleep and the sleep EEG were found following three months of chronic DLAN exposure. Particularly, in all vigilance states prominent frequencies in the EEG showed a general attenuation. Additionally, by conducting detrended fluctuation analysis on the locomotor activity data, a significantly less healthy rest-activity pattern was found following three months of continuous DLAN exposure. Taking into account the behavioral, sleep and the sleep EEG parameters, the data suggest that chronic DLAN exposure impacts the sleep regulatory system and brain integrity, diminishing its adaptability and reactivity.

Keywords: sleep, dim-light-at-night (DLAN), electroencephalogram, detrended-fluctuation-analysis, sleep deprivation

Introduction

Artificial light has a widespread use in modern society. In addition to its benefits, 'light pollution' is an increasing impediment in society, i.e. light levels in the evening or night exceed natural light levels [1, 2]. Light exposure at night has been associated with various health disruptions including metabolic and immunological disturbances, as well as altered circadian timing [3]. The latter could evoke a multitude of downstream effects that consequently impacts sleep [4, 5, 6].
Sleep is coordinated by a circadian process, which is regulated by the biological clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus, and a sleep homeostatic process, which is dependent on prior waking and sleep duration [7, 8]. In mammals, the homeostatic sleep process is considered to be reflected in the NREM sleep electroencephalographic (EEG) slow-wave activity (SWA, EEG power density between 0.75-4.0 Hz) [7, 8]. Sleep deprivation has been experimentally used to test the sleep homeostatic process and investigate sleep characteristics under elevated sleep pressure conditions [8]. Whole night bedside light of 40 lux was demonstrated to have detrimental effects on sleep quality in humans including frequent arousals, decreased slow-wave sleep and reduction of SWA and activity in the spindle frequency bands during NREM sleep and theta frequency bands during REM sleep [9]. Dim-light-at-night (DLAN, 5 or 10 lux) was shown to increase waking after sleep onset, NREM sleep stage 1 and REM sleep, whereas NREM sleep stage 2 was decreased in human subjects [10]. DLAN of 5 lux was shown to disrupt molecular circadian rhythms and increase body weight in mice [11], disturb the daily sleep-wake cycle in the rat [12], and modify the expression of daily rhythms and behavioral patterns in the grey mouse lemur [13]. However, a recent study in mice showed an absence of effects of DLAN on timing as well as quality of sleep in mice [14] and, hence, more data are needed before a consensus can be reached on the effect of DLAN on sleep.
In the present study, we investigated the effect of exposure to different DLAN duration periods, i.e. one day, one week, one month and three months, on rest-activity behavior, sleep architecture, and sleep EEG parameters in C57BL/6J mice. Sleep architecture under DLAN exposure was characterized by a gradual delay of vigilance state rhythms in the course of prolonged exposure. Following three months of DLAN exposure, SWA and its 24-h rhythm was considerably attenuated, and EEG spectral alterations emerged in all vigilance states. Detrended-fluctuation analysis on the locomotor activity data [15] indicated overall a less healthy physiology. Our data suggest that not only behavioral rhythmicity and sleep, but also brain integrity and consequently general health are compromised by chronic DLAN exposure.

Materials and methods

Animals

Young adult male C57BL/6JOlaHSD mice (n=14) (Harlan, Horst, the Netherlands) were used for this study. A group of mice (n=5) was exposed to DLAN for one month in total. A second group (n=9) was exposed to DLAN for continuous three months. The age of all mice at the time of the recordings was six months. The animals were individually housed

under controlled conditions [12:12 h light:dark cycle (75lux:0lux) or 12:12 h light:DLAN (75lux:5lux); lights on at 10:00] with food and water available ad libitum in a temperature controlled room (21-22°C).
All animal experiments were approved by the Animal Experiments Ethical Committee of the Leiden University Medical Centre (the Netherlands) and were carried out in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Surgeries

Under deep anesthesia (Ketamine 100 mg/kg; Xylazine 10 mg/kg; Atropine 1 mg/kg), EEG recording screws (placed above the somatosensory cortex and cerebellum) and electromyogram (EMG) electrodes (placed on the neck muscle) (Plastics One) were implanted as described previously [16, 17, 18, 19]. The wire branches of all electrodes were set in a plastic pedestal (Plastics One, Roanoke, VA) and fixed to the skull with dental cement. The mice were allowed to recover for 7-10 days.

Light schedules and behavioral recordings

Mice were kept on a 12:12 h Light:Dark schedule. At the age of five-six months, mice (n=5) were implanted with EEG and EMG electrodes, as aforementioned. In order to investigate the effect of DLAN on behavior, vigilance states and the EEG, sleep was recorded after one control day of sleep-wake recordings in light/dark conditions (Light: 75 lux, Dark: 0 lux) (control LD), after one day (1d DLAN), one week (1w DLAN) and one month (1m DLAN) exposure to 12:12 h Light:DLAN (75:5 lux) (Fig. 1a). An additional group of mice (n=9) was exposed for 12 weeks to 12:12h Light:DLAN (3m DLAN) starting at the age of three months. Subsequently, EEG and EMG electrodes were implanted and one week later the animals were recorded (Fig. 1a). At the time of the recordings the age of all mice was approximately six months. Locomotor activity for the control light-dark, the one-month and three-month conditions was monitored by passive infrared detectors (PIR, Actimetrics software, Wilmette, Il, USA) prior to the EEG/EMG recordings. For these conditions, the rhythm period and strength was determined by F-Periodogram analysis, as described earlier [12, 20].

Assessment of scale invariance using Detrended Fluctuation Analysis (DFA)

To assess the scale-invariant patterns in behavior for the mice under different conditions (control LD, 1m and 3m DLAN), we use DFA in order to calculate the correlations present in the fluctuations in behavioral activity patterns at different timescales [21, 22]. In short, a DFA calculates an averaged fluctuation value F(n) for a certain window size n. The window sizes are increased in a logarithmic manner, and for each window size a fluctuation value is determined. Then the fluctuation values F(n) are plotted against window sizes n on a log-log scale. A straight line means that there is scale-invariance in the data in the range of window sizes for which the straight line holds. The angle α can be calculated for each stretch of straight line. This value α is the scaling exponent for the range of time scales indicated by the stretch of straight line and it signifies the correlation properties in the signal. The same 15-day range that was used in analyzing

the rest-activity data for period and rhythm strength was also used to perform the DFA analysis. The circadian range for DFA analysis is from 3h up to 24h [22]. As our data above 8h are not statistically reliable due to restriction of data length [23], we used the range from 3-8 h to determine α as the circadian range.

EEG recordings

The EEG and EMG were recorded with a portable recording system (PS 1 system, Institute of Pharmacology and Toxicology, Zurich, Switzerland) as previously described [16, 17, 18, 19, 24]. Before each recording, a calibration signal was recorded on the EEG and EMG channels. Both signals were amplified, conditioned by analogue filters and sampled at 512 Hz. The signals were filtered through a digital finite impulse response filter and stored with a resolution of 128 Hz. EEG power spectra were computed for 4-s epochs by a FFT routine within the frequency range of 0.25-25.0 Hz. To record the EEG and EMG, animals were placed into experimental chambers and connected through a flexible cable and a counterbalanced swivel system to the recording setup. Conditions in the experimental chamber were similar to the home cage, including light conditions, food and water availability. Before starting each experiment, animals were allowed to adjust to the experimental conditions for a week. Subsequently, a 24-h light-dark period was recorded (control LD), starting at lights on, followed by 12-h light and 12-h DLAN (acute DLAN, 1d DLAN) (schematic overview of experimental design in Figure 1a). For the four experimental conditions, namely 1d DLAN, 1w DLAN, 1m DLAN and 3m DLAN, first a baseline day (BL, 12L:12DLAN) was recorded, followed by a second day in the same light/DLAN schedule, at the start of which six hours of sleep deprivation were conducted by gentle handling [16, 17, 18, 19, 25]. EEG and EMG were recorded continuously during BL and sleep deprivation, as well as for 18 hours of recovery period.

Data analysis and statistics

Three vigilance states (Waking, NREM sleep and REM sleep) were scored offline in 4s epochs by visual inspection of the EEG and EMG signals as well as EEG power density in the slow-wave range, as described previously [16, 17, 18, 19, 25]. For each epoch, the EEG power density in the delta (0.75-4.0 Hz) and theta band (6.25-9.0 Hz) and the integrated EMG value were graphically displayed on a PC monitor to enable scoring of the different vigilance states. The vigilance states were expressed as a percentage of artifact-free recording time. Epochs with artifacts were excluded from the subsequent data analysis of the power spectra, but vigilance states could always be determined. To test whether DLAN altered the daily vigilance state distribution a two-way ANOVA was performed (factors 'treatment' and 'time of day') on 6-h and 12-h mean values of vigilance states. In addition, a two-way ANOVA (factors 'treatment' and 'time of day') was performed in order to detect differences in the baseline (BL) time course of 2-h vigilance states values and EEG power density spectra in NREM sleep, between the different DLAN conditions and the control LD. Three-way ANOVAs were performed to investigate potential sleep alterations across different DLAN duration periods, across BL and after sleep deprivation, on 2-h and 12-h mean vigilance states values and EEG power density values in NREM sleep (factors 'treatment', 'Light-Dark', 'time of day', 'day'). Note that the 6-h value after sleep deprivation is compared to the corresponding 6-h value

of BL. A two-way ANOVA was performed on the 24-h EEG power density values of the three vigilance states with main factors 'treatment' and 'EEG frequency bins'. One-way ANOVAs (factor 'treatment', 'period', 'strength') were performed to test potential differences in 24-h vigilance state amount and in rest-activity data across all groups. When appropriate, paired and unpaired post-hoc Bonferroni-corrected student's t-tests were applied to determine the effects of treatment and sleep deprivation.

Results

Rest-activity behavioral data and DFA analysis

An overview of the experimental design is shown in Figure 1a (see more details in *Materials and Methods*). Examples of representative rest-activity behavior from animals exposed to control LD, one and three months DLAN conditions are shown in Figure 1b (15 days). F-periodogram analysis showed that the strength of the behavioral rhythm was substantially decreased following three months DLAN exposure as compared to control LD, and intermediate results were obtained following one month DLAN exposure, both one and three month DLAN groups being significantly different from control LD (Mean $\pm$ SD, Control LD: 0.21 $\pm$ 0.02, 1m DLAN: 0.14 $\pm$ 0.049, 3m DLAN: 0.11 $\pm$ 0.03, One-way ANOVA, factor 'period': $p<0.0001$, post-hoc Bonferroni multiple comparisons correction t-tests between groups).
Applying DFA on the behavioral data (more details in Materials and Methods), we found the scaling component α to be significantly lower in animals exposed to both one and three months DLAN compared to control LD (One-way ANOVA, factor 'group' $p=0.023$, post-hoc Bonferroni multiple comparisons correction t-tests between groups) (Fig. 2).

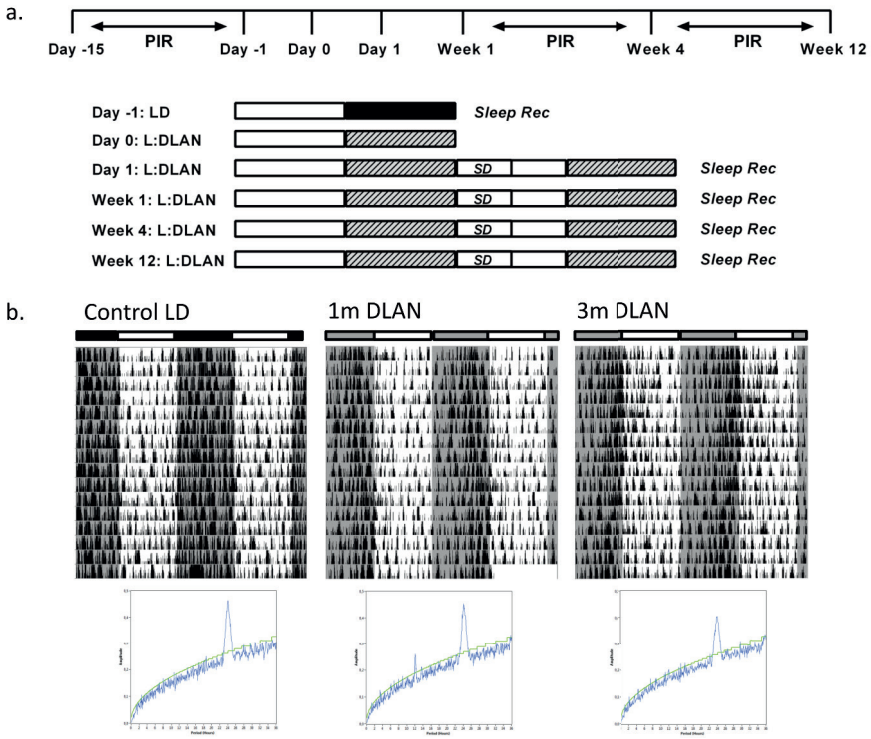

Figure 4.1: a. Schematic overview of the experimental design. Sleep was recorded during the control day (Day -1) in 12:12h Light (75 lux):Dark (0 lux) (LD) (n=5). After one night of 12-h dim light, one week, one month (n=5) and three months (n=9) of continuous exposure to 12:12h Light:Dim-light-at-night (DLAN: 5 lux), sleep recordings were repeated consisting of a baseline 24-h day followed by 6-h sleep deprivation and 18-h recovery period. Passive infrared detectors (PIR) were inserted in the cages for behavioral recordings during 15 days of control LD condition, one and three months of continuous exposure to 12:12h Light:DLAN.
b. Representative double-plotted actograms of 3 mice exposed to control light-dark (LD, 75:0lux), one month and three months 12:12h light:dim-light-at-night (DLAN, 75:5lux) with the corresponding F-periodogram analysis (Mean $\pm$ SD: *Circadian period*, Control LD: 24.0h $\pm$ 0.0, 1m DLAN: 24.4h $\pm$ 0.35, 3m DLAN: 24.08h $\pm$ 0.21, more details in the text).

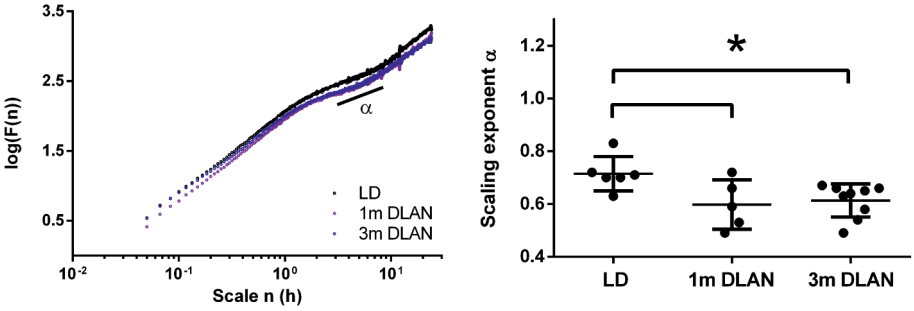

Figure 4.2: Scale-invariant correlation of behavioral activity fluctuations based on 15 days of behavioral data. Representative examples of three mice from the three different experimental groups (left panel). The scale-invariant component alpha at timescale from 3-8h (right panel) is plotted (mean ± SD) for the control light-dark group (LD), the 1 month and 3 months dim-light-at-night (DLAN) groups. Asterisks indicate significant differences between the groups ($p<0.05$, two-tailed unpaired t-tests).

Sleep architecture

In the course of DLAN exposure, waking increased and NREM sleep decreased during the light period (L1). This effect was significant after 3 months DLAN exposure, compared to control LD (Fig. 3) (post-hoc two-tailed unpaired t-tests with Bonferroni multiple comparisons correction, *Supplementary Material* Table S1). No significant changes were evident in the 12-h dark/dim light period (D1) and in 24-h vigilance state values among groups ($p>0.05$). The day-night amplitude was found to be decreased in 1w, 1m and 3m DLAN groups compared to LD and 1d DLAN groups, although this decrease was not significant (*Supplementary material*, Fig. S1). In the 6-h of the light period after sleep deprivation (L2), mice exposed to 3 months DLAN showed more waking and less NREM sleep compared to mice exposed to 1 day, 1 week and 1 month of DLAN, whereas no differences were found in the following dim light period (D2) (Fig. 3) (post-hoc two-tailed unpaired t-tests with Bonferroni multiple comparisons correction, *Supplementary Material* Table S1). Compared to BL, sleep deprivation increased NREM sleep in the light period and decreased waking in both periods (light and dim light: L2, D2) in the 1 week DLAN group and increased REM sleep in the dim light period in the 1 week, 1 month and 3 months DLAN groups (post-hoc two-tailed paired t-tests between BL and after sleep deprivation time, *Supplementary Material* Table S1).

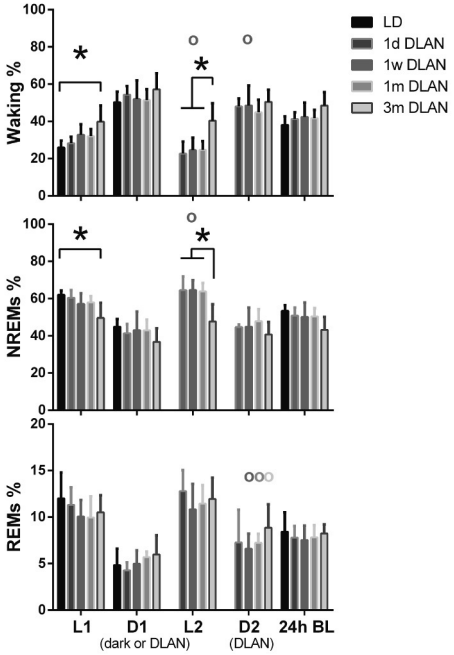

Figure 4.3: Distribution of each behavioral state (Waking, NREM and REM sleep) during the baseline day (BL) and after sleep deprivation. Bar plots represent mean (± SD) values [L1, D1, D2 correspond to 12-h values and L2 to 6-h values for the recovery period after sleep deprivation, for light and dark/dim light periods during the 48-h recordings respectively] and 24-h values of baseline recordings (24-h BL) for Waking, NREM and REM sleep for control (LD), one day, one week, one month (n=5) and three months (n=9) dim-light-at-night (DLAN) conditions. Asterisks indicate significant differences between the groups and circles indicate significant differences between recovery and BL day (post-hoc unpaired and paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment', 'Light-Dark', 'day').

Additionally, vigilance state distribution over the 24-h BL day was changed in the course of DLAN exposure, showing a gradual delay in the waking peak in the dim light period after 1 day, 1 week and 1 month of DLAN exposure compared to control dark (Fig. 4) (post-hoc two-tailed unpaired t-tests with Bonferroni multiple comparisons correction, *Supplementary Material* Table S1). In the course of DLAN exposure, up to 1 month, the time in the dark/dim light period of the waking peak was positively correlated with the prior duration of DLAN exposure ($R^2 = 0.70$) (Fig. S2). After 3 months DLAN exposure, mice showed a decrease in NREM sleep during the light period, while waking and NREM sleep levels did not differ from control dark period in the first half of the dim light period and no clear waking peak was evident in the dim light period.

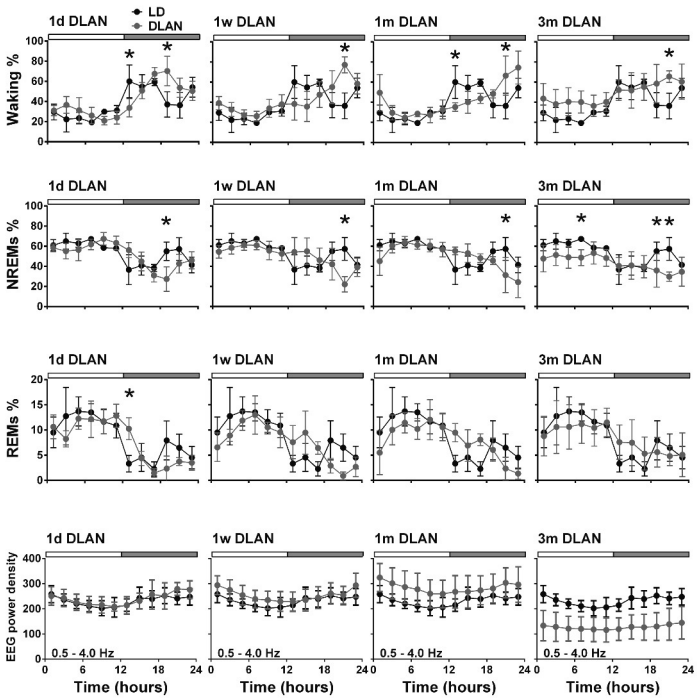

Figure 4.4: Time course of vigilance states and EEG power density in NREM sleep for frequencies 0.5-4.0 Hz (slow-wave-activity), during 24-h baseline for control light:dark (LD), one day, one week, one month (n=5) and three months (n=9) dim-light-at-night (DLAN) conditions. Curves connect 2-h mean (± SD) values of Waking, NREM and REM sleep as well as EEG power density in NREM sleep. The black curves signify LD conditions, while the grey curves DLAN conditions. The control day is plotted in each condition for easy comparison. The white and grey bars above each graph indicate the light-dark/dim light cycle (rest-active period respectively). Black asterisks at the top of each graph represent significant differences between the control LD and the different DLAN duration period conditions across the 24-h period (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVAs, main effects 'treatment', 'time of day'). Significant different EEG power levels from the control LD condition were obtained in the 1-month and 3-month DLAN groups, and 1 month and 3 month DLAN groups differed significantly from all other groups.

Throughout the day-night cycle, changes seemed to prevail in the first and second half of the dark period, whereas no large alterations were found in the light period. To investigate this further, we calculated the 6h values in the dark/dim light period. Effects that reflect the delay in waking were found in the first and second half of the dark/dim light period (D1.1 and D1.2) (Fig. S3) (post-hoc two-tailed unpaired t-tests with Bonferroni multiple comparisons correction, *Supplementary Material* Table S1). In particular, in the first 6h of the dim light period, waking was significantly decreased after 1 week and 1 month DLAN exposure, whereas REM sleep was increased after 1 month DLAN compared to control. In the second 6h of the dim light period, waking significantly increased and NREM sleep decreased after 1 week, 1 month and 3 months DLAN exposure, com-

pared to the control dark period.

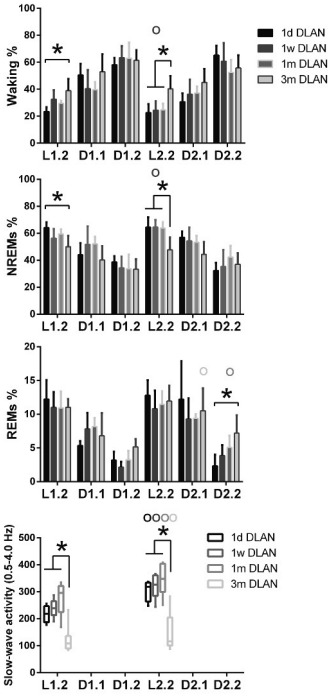

Figure 4.5: Distribution of each behavioral state (Waking, NREM and REM sleep) and slow-wave activity in NREM sleep (SWA, EEG power between 0.5-4 Hz) during baseline (BL) and during corresponding time intervals after sleep deprivation. Bar plots represent mean (± SD) 6-h values for light and dark/dim light periods and boxplots of SWA the first 2-h after sleep deprivation with the corresponding time in BL for control (LD), one day, one week, one month (n=5) and three months (n=9) dim-light-at-night (DLAN) conditions. L1.2, D1.1, D1.2, L2.2, D2.1, D2.2 correspond to 6-h values for light and dark/dim light periods during BL and following sleep deprivation. Asterisks indicate significant differences between the groups and circles indicate significant differences between recovery and BL day (post-hoc unpaired and paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment', 'time of day', 'day').

Across increasing DLAN duration periods, significant differences were found between 1 day and 1 week and 1 month groups in the 2-h distribution of vigilance states (*Supplementary Material*, Fig. S4). These consisted of more waking and less NREM and REM sleep at the beginning of the dim light period in the 1d DLAN group compared to the 1m DLAN, contrary to the end of the dim light period in which more NREM sleep and less waking was found in the 1d DLAN group compared to both the 1w and 1m DLAN groups (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1). Following sleep deprivation, the differences in the vigilance states were more pronounced between the 3 months DLAN group and the shorter DLAN exposure groups in

which less NREM sleep and more waking was apparent in the 3 months DLAN group in the six hour light period after sleep deprivation (L2.2) and more REM sleep in the last six hours of the dim light period after sleep deprivation (D2.2) (Fig.5 and in more detail in the 2-h values in *Supplementary material*, Fig. S4). Compared to BL, mice exposed to short DLAN duration periods showed alterations in the amount of waking, NREM and REM sleep, while the 3 months DLAN exposure group showed no changes after sleep deprivation (*Supplementary Material*, Fig. S4).

EEG power density

The waking, NREM and REM sleep EEG power density spectra values were lower in the mice exposed to 3m DLAN, becoming significant in the slow-wave (1-4 Hz) and theta frequencies (5-9 Hz) compared to the control LD and the other DLAN conditions (Fig. 6) (post-hoc two-tailed unpaired t-tests with Bonferroni multiple comparisons correction), indicating that in each vigilance state the most prominent frequencies in the EEG showed a reduced power after 3 m DLAN exposure.
EEG power in NREM sleep between 0.5-4.0 Hz (EEG SWA) was altered across groups (Fig. 4, lowest panel) (post-hoc Bonferroni multiple comparisons correction t-tests between groups in the overall period of 24-h, *Supplementary Material* Table S1). Compared to the control LD group, 1 week and 1 month DLAN exposure showed a small and gradual increase in EEG SWA during NREM sleep. In accordance with the general decrease found in the EEG power density, the 3m DLAN group had the lowest EEG SWA levels. Significant different levels from the control LD condition were obtained in the 1-month and 3-month DLAN groups, whereas the 1 day and 1 week DLAN conditions did not differ from the LD control group. Additionally, 1 month and 3 month DLAN groups differed significantly from all other groups. These effects are also evident in the first 2-h following sleep deprivation, as well as in the corresponding 2-h period in BL (Fig.5, lower panel) (post-hoc Bonferroni multiple comparisons correction t-tests between groups in the 2-h light period, *Supplementary Material* Table S1). Sleep deprivation induced an increase in SWA in all groups (Fig. 5 and *Supplementary Material* Fig. S4) (post-hoc Bonferroni multiple comparisons correction paired t-tests between BL and after sleep deprivation time, *Supplementary Material* Table S1).

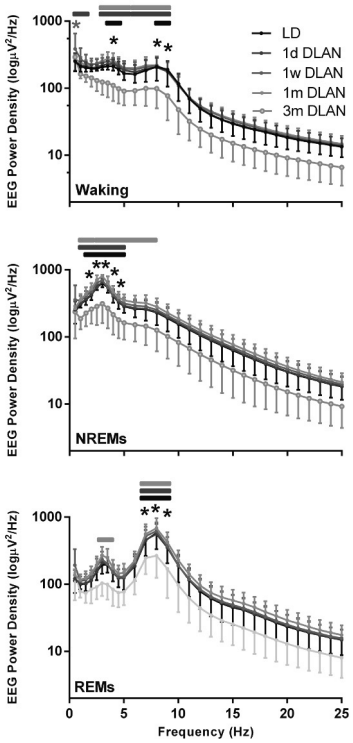

Figure 4.6: EEG power density in Waking, NREM and REM sleep for control light-dark (LD), one day, one week, one month (n=5) and three months (n=9) dim-light-at-night (DLAN) conditions during 24-h baseline (Mean ± SD) (0.5-25 Hz). Black and gray asterisks indicate significant differences between the control LD and the 3 months and 1 week DLAN groups respectively and black, dark gray and light gray lines indicate significant differences between the 3 months DLAN and the 1 day, 1 week and 1 month DLAN respectively (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment', 'EEG frequency bin').

Discussion

In the present study, we found that rest-activity, vigilance state rhythms, and EEG parameters deteriorate in the course of DLAN exposure. Altered sleep parameters were evident after merely 12-h DLAN exposure and considerable consequences on locomotor activity, sleep behavior, and the EEG were found after chronic DLAN exposure. Regarding the sleep architecture, most alterations were apparent during the active period, with the waking peak being delayed following 1d to 1m DLAN conditions compared to control LD, suggesting a general delay of the main sleep and waking period occurring in the course of prolonged DLAN exposure. Moreover, after 3 months of DLAN exposure, overall increased waking and decreased NREM sleep was found during the (normally) inactive light period. Notably, SWA in NREM sleep was differentially altered in the course of

DLAN exposure. In the course of 1w and 1m DLAN, it was gradually increased compared to control LD, whereas after 3m of DLAN exposure it was greatly attenuated, below control LD levels. In parallel, a general decrease in prominent frequencies (slow-waves and/or theta) of all vigilance states was found after 3 months DLAN exposure. Regarding the behavioral analysis, DLAN exposure induced a delay in the activity onset in the dim light period and a gradual decrease in the strength of the rhythm after 1 and 3 months. Interestingly, DFA on the rest-activity data revealed a decrease in the scaling component suggesting attenuated adaptability and integrity of the circadian system. Our data show that long-term DLAN exposure can negatively impact sleep and daily rhythms influencing the overall physiology and behavior.

Sleep architecture

An important aspect of the effect of DLAN on vigilance states distribution is the delay of the daily maximum of the active waking period in the dim light period, compared to control LD dark period. This delay lasted from 2-8 hours and increased as a function of DLAN exposure from 1 day to 1 month (Fig. 4 and Fig. S2). Although the dynamics of the daily light-dark/dim amplitude were not significantly disturbed following 1d, 1w, 1m of DLAN exposure, they seem to become distorted after 3 months DLAN exposure. In this group, waking was increased during the light period. Similar to the delayed activity and waking peak, delayed temperature rhythms were noted after 2 weeks of DLAN exposure in the grey mouse lemur [13]. A recent study in Wistar rats, also showed a gradual reduction in rhythm amplitude in vigilance state distribution, starting from day 1 to day 14 of DLAN exposure [12]. Our data are in accordance with Stenvers et al study (2016), however many effects in our mice occurred at a later time point. As our light levels were very similar this suggests that longer exposure to the DLAN condition was required before effects were visible in the mice. Stenvers et al (2016) performed their study in albino rats, whereas our study was conducted in pigmented mice, which may explain this difference in duration. In general, pigmented animals are less susceptible to light compared to albino animals [26, 27]. In contrast, in a study in Swiss Webster mice, long-term DLAN exposure did not induce any alterations in sleep architecture [14]. However, in that study, in addition to an eight-week DLAN exposure, a running wheel was available in the cage. Introduction of a running wheel for mice, that can be used long-term voluntarily on a daily basis, has been shown to substantially affect sleep and the sleep EEG [28, 29]. Interestingly, we recently shown that voluntary use of a running wheel on a daily basis is able to improve sleep architecture and the sleep EEG of mice substantially, lasting for at least two weeks after removal of the wheel [19]. In the current study, we show that in a DLAN condition in animals that do not receive this cage enrichment, quality of sleep will deteriorate and the distribution of sleep will become more random, but at a slower rate than seen in albino rats.

In order to test the sleep homeostatic response after elevated sleep pressure, we conducted a 6-h sleep deprivation in all DLAN groups [16, 17, 18, 19, 25]. Sleep deprivation induced differences among DLAN groups, separating the 3m DLAN from the other groups, which showed more waking and less NREM, during the 6-h of the recovery light period (Fig. 3, 5). Although mice exposed to all DLAN durations were sleep deprived equally successful, it seems that sleep deprivation did not restore behavior during the subsequent

recovery period, as if the mice were less susceptible to elevated sleep pressure. Only mice exposed to 1w DLAN showed the normal increase in NREM sleep during the 6-h recovery light period after sleep deprivation (Fig. 3, 5). Consistent between DLAN durations was the increased REM sleep, particularly in the first part of the active period after sleep deprivation in the 1w to 3m DLAN conditions.

Rest-activity behavior and fractal patterns

In order to decipher the impact of DLAN exposure on daily rhythms, we conducted additional analysis on the rest-activity data. DFA has been used in many studies to quantify correlations in physiological time series [30]. With DFA information enclosed in physiological data reveals the potential effects of network fluctuations at different timescales. As demonstrated by a large body of literature [31, 32], scale-invariant correlations with $\alpha \sim 1$ are associated with healthy physiology, which is not too responsive to external perturbations, but flexible enough to adapt to these external perturbations (adaptive, but not reactive). In our study, DFA revealed an α close to 1 in the control LD condition and α decreased as a function of DLAN duration. Most healthy physiological systems show scaling components around 1, whereas deviations from 1 are associated with disease or aging [15, 22]. The pattern of alterations as indicated by the fractal view of physiology likely describes underlying mechanisms of network-wide responsiveness and adaptability.
Exposure to DLAN is likely to influence functioning of the circadian clock in the SCN. The SCN appears to have regulatory functions at multiple time scales, which exceed the conception of the traditional circadian time with values around 24 hour [33]. We are showing and using the 3-8 h timescale which is known to be influenced by the circadian clock [34]. In the course of prolonged exposure to DLAN, mice in our study exhibit a gradual decrease in their α values. This shows that DLAN, particularly when it is chronic, can attenuate daily fluctuations corresponding to healthy physiology. In accordance, following a long-term DLAN protocol of four weeks, it was shown that Swiss Webster mice increased their percentage of food consumed especially during the light period, and revealed disrupted molecular circadian rhythms during the active period [11]. Our data suggest that the complex network of the brain that regulates circadian behavior is less adaptable to perturbations and its integrity is negatively affected by DLAN [30].

EEG power density in waking, NREM and REM sleep and slow-wave activity (SWA) in NREM sleep

The EEG is a window on the brain, and alterations in the EEG can be interpreted in the context of neuronal network integrity and adaptability [17, 35]. Spectral analysis of the EEG revealed that in waking, NREM and REM sleep, mice exposed to 3 months DLAN had significant differences with control LD as well as the 1d, 1w, and 1m of DLAN. Particularly, mice that were exposed to 3m DLAN showed a general decrease in slow delta activity in NREM sleep and theta frequencies activity in REM sleep and waking compared to all the other experimental groups. SWA levels in NREM sleep showed similar patterns, being slightly increased across 48-h in short- and mid-term DLAN exposure up to 1 month, however, demonstrating a great general decrease after 3 months of DLAN exposure. In accordance with the attenuated rhythmicity in rest-activity after 3 months

DLAN exposure, the spectral alterations suggest a reduced quality of brain cortical activity in all three vigilance states. The subtle decrease in slow-wave activity in the waking spectra after 3 months DLAN exposure suggests that these mice are less drowsy [36, 37]. However, the concomitant more robust decrease in waking theta, indicates that these animals are also less active and alert [36, 37]. These are two contradicting findings, and therefore these data suggest that brain integrity in general may be affected by long-term DLAN exposure and that this is reflected in the EEG.
In humans, nocturnal light exposure of 40 lux had similar effects on the sleep EEG, where slow, delta and spindle frequency activity in NREM sleep as well as theta activity in REM sleep was decreased [9]. In addition, rats exposed to 14 days of DLAN, showed decreased SWA during the light period, increased SWA at the end of the active period and a general decrease in EEG activity between 16-19 Hz during NREM sleep [12]. In contrast, Swiss Webster mice showed no alteration in SWA after eight weeks of DLAN exposure compared to control LD. However, in the latter study the daily modulation in SWA was also lacking in the control LD group [14]. Although the increase in NREM sleep after sleep deprivation was attenuated, after DLAN exposure, all DLAN groups showed an increase in SWA levels during the first 2h after sleep deprivation. This shows that, although the amount of NREM sleep did not always increase, part of the homeostatic sleep response, expressed in NREM sleep SWA, was still functioning [7, 8].
As aforementioned, the deterioration of all prominent EEG frequencies across the three different vigilance states could imply attenuation in the EEG generation caused by alteration in the neuronal network underneath. Rhythmic events within the delta frequency range are intrinsically generated in thalamic neurons [38]. However, this activity cannot reach the cortex to be reflected at the macroscopic EEG level, unless thalamic neurons are synchronized [38]. Thalamo-cortical projections underlie the performance of specific functional tasks during alert as well as sleep states. Therefore, the general decrease in EEG power density in the low frequencies may be caused by a loss of thalamocortical synchronization due to prolonged DLAN exposure. Furthermore, since the cerebral cortex is not merely a passive receiver of synchronized delta potentials of thalamic origin, but these inputs are reorganized by the intrinsic properties and synaptic events in cortical circuits [38, 39], alterations following DLAN exposure may also take place at the level of the cortex.
In short, chronic DLAN exposure induces a reduction of the slope α in the DFA analysis of locomotor activity, indicating a less adaptive and potentially a more reactive system. In addition, sleep and EEG data suggest an overall altered thalamo-cortical neuronal network. When challenged with a perturbation, in this case a sleep deprivation, the response is inadequate as the increase in NREM sleep is attenuated. Therefore, the data suggest that the dynamics of the underlying sleep regulatory mechanisms are altered and that the integrity of the brain network is distorted following chronic DLAN exposure.
The benefits of electricity and electric lighting are indisputable. Nevertheless, recent research associates light exposure at night with health consequences. The first deleterious effects regarding sleep in our study are apparent after one night of DLAN exposure. Prolonged exposure to DLAN induces additional rest-activity rhythm disruptions together with further sleep and EEG quality degradation. The scale invariant component of DFA is reduced indicating unhealthy correlations under DLAN that can possibly render the organism not able to adjust to altered environmental conditions, which is confirmed by

the results of the sleep deprivation experiment. Concluding, DLAN induces a diminished integrity of the circadian, sleep, and EEG regulatory systems and probably has additional effects on neurophysiological systems, impacting sleep health as well as the underlying brain network.

Bibliography

[1] Smith FG (1979) Report and recommendations of IAU commission 50, reports on astronomy. IAU Transactions XVIIA: 218-222.

[2] Cinzano P, Falchi F, Elvidge CD (2001) The first world atlas of the artificial night sky brightness. Monthly Notices of the Royal Astronomical Society 328: 689-707.

[3] Navara KJ, Nelson RJ (2007) The dark side of light at night: physiological, epidemiological, and ecological consequences. J Pineal Res 43: 215-224.

[4] Duffy JF, Kronauer RE & Czeisler CA (1996) Phase-shifting human circadian rhythms: influence of sleep timing, social contact and light exposure. J Physiol 495 (Pt 1):289-297.

[5] Czeisler CA & Wright KP Jr (1999) Influence of light on circadian rhythmicity in humans. I. Regulation of Sleep and Circadian Rhythms, eds. Turek FW & Zee PC (Marcel Dekker, Inc., New York) pp. 149-180.

[6] Zeitzer JM, Dijk DJ, Kronauer R, Brown E, Czeisler C (2000) Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. J Physiol 526 (Pt 3): 695-702.

[7] Borbély AA, Daan S, Wirz-Justice A, Deboer T. (2016) The two-process model of sleep regulation: a reappraisal. J Sleep Res 25(2):131-143.

[8] Achermann P. & Borbély AA. (2017). Sleep homeostasis and models of sleep regulation in Principles and Practice of Sleep Medicine, eds. Kryger MH, Roth T & Dement WC), (Elsevier), pp. 377-387.

[9] Cho JR, Joo EY, Koo DL, Hong SB. (2013) Let there be no light: the effect of bedside light on sleep quality and background electroencephalographic rhythms. Sleep Med 14(12):1422-1425.

[10] Cho CH et al. (2016) Exposure to dim artificial light at night increases REM sleep and awakenings in humans. Chronobiol Int 33(1):117-123.

[11] Fonken LK, Aubrecht TG, Meléndez-Fernández OH, Weil ZM, Nelson RJ. (2013) Dim light at night disrupts molecular circadian rhythms and increases body weight. J Biol Rhythms. 28(4):262-271.

[12] Stenvers DJ et al. (2016) Dim light at night disturbs the daily sleep-wake cycle in the rat. Sci Rep 6:35662.

[13] Le Tallec T, Perret M, Théry M. (2013) Light pollution modifies the expression of daily rhythms and behavior patterns in a nocturnal primate. PLoS One 8(11):e79250.

[14] Borniger JC, Weil ZM, Zhang N, Nelson RJ. (2013) Dim light at night does not disrupt timing or quality of sleep in mice. Chronobiol Int. 30(8):1016-23.

[15] Peng CK, et al. (1995) Fractal mechanisms and heart rate dynamics. Long-range correlations and their breakdown with disease. J Electrocardiol 28(Suppl):59-65.

[16] Deboer T, van Diepen HC, Ferrari MD, Van den Maagdenberg AMJM & Meijer JH (2013) Reduced sleep and low adenosinergic sensitivity in Cacna1a R192Q mutant mice. Sleep 36(1): 127-136.

[17] Panagiotou, M., Vyazovskiy, V.V., Meijer, J.H. & Deboer, T. (2017). Differences in electroencephalographic non-rapid-eye movement sleep slow-wave characteristics between young and old mice. Sci Rep 7:43656.

[18] Panagiotou, M., Meijer, J.H., Deboer, T. (2018) Chronic high-caloric diet modifies sleep homeostasis in mice. Eur J Neurosci 47(11):1339-1352.

[19] Panagiotou M, Papagiannopoulos K, Rohling JHT, Meijer JH & Deboer T (2018) How old is your brain? Slow-wave activity in non-rapid-eye-movement sleep as a marker of brain rejuvenation after long-term exercise in mice. Front Aging Neurosci (in press) doi: 10.3389/fnagi.2018.00233.

[20] Jenni, O.G., Deboer, T. and Achermann, P. (2006) Development of the 24-h rest-activity pattern in human infants. Infant Behav Dev 29(2):143-152.

[21] Peng, C.K. et al. (1994) Mosaic organization of DNA nucleotides. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics 49(2):1685-1689.

[22] Gu C. et al. (2015) Lack of exercise leads to significant and reversible loss of scale invariance in both aged and young mice. Proc Natl Acad Sci U S A 112(8):2320-2324.

[23] Hu K, Ivanov PC, Chen Z, Carpena P, Stanley HE. (2001) Effect of trends on detrended fluctuation analysis. Phys Rev E 64(1 Pt 1):011114.

[24] Deboer, T., Ruijgrok, G. & Meijer, J.H. (2007) Short light–dark cycles affect sleep in mice. Eur J Neurosci 26(12):3518-3523.

[25] Huber R, Deboer T & Tobler I (2000) Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. Brain Res 857(1-2): 8-19.

[26] Tobler I, Franken P, Alfoldi P, Borbély AA. (1994) Room light impairs sleep in the albino rat. Behav Brain Res 63(2):205-211.

[27] Benca RM, Gilliland MA, Obermeyer WH. (1998) Effects of lighting conditions on sleep and wakefulness in albino Lewis and pigmented Brown Norway rats. Sleep 21(5):451-460.

[28] Lancel M, Droste SK, Sommer S, & Reul JM (2003) Influence of regular voluntary exercise on spontaneous and social stress-affected sleep in mice. Eur J Neurosci 17(10): 2171-2179.

[29] Vyazovskiy, V.V., Ruijgrok, G., Deboer, T. and Tobler, I. (2005) Running wheel accessibility affects the regional electroencephalogram during sleep in mice. Cereb. Cortex 16(3):328-336.

[30] Pittman-Polletta BR, Scheer FA, Butler MP, Shea SA, Hu K. (2013) The role of the circadian system in fractal neurophysiological control. Biol Rev Camb Philos Soc 88(4):873-894.

[31] Goldberger AL et al. (2002) Fractal dynamics in physiology: alterations with disease and aging. Proc Natl Acad Sci U S A 99 (Suppl 1):2466-2472.

[32] Hardstone, R. et al. (2012) Detrended fluctuation analysis: a scale-free view on neuronal oscillations. Front Physiol 3:450.

[33] Hu, K. et al. (2012) Fractal patterns of neural activity exist within the suprachiasmatic nucleus and require extrinsic network interactions PLoS One 7(11):e48927.

[34] Hu K, Van Someren EJ, Shea SA, Scheer FA. (2009) Reduction of scale invariance of activity fluctuations with aging and Alzheimer's disease: Involvement of the circadian pacemaker. Proc Natl Acad Sci U S A 106(8):2490-2494.

[35] Vyazovskiy VV, Cirelli C, Tononi G. (2011) Electrophysiological correlates of sleep homeostasis in freely behaving rats. Prog Brain Res 193:17-38.

[36] Vanderwolf CH (1992) The electrocorticogram in relation to physiology and behavior: a new analysis. Electroencephalogr Clin Neurophysiol 82:165-175.

[37] Huber R, Deboer T & Tobler I (1999) Prion protein: a role in sleep regulation? J Sleep Res 8(Suppl 1): 30-36.

[38] Steriade M, Dossi RC, Nuñez A. (1991) Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically induced synchronization and brainstem cholinergic suppression. J Neurosci 11(10):3200-3217.

[39] Steriade M, Nuñez A, Amzica F. (1993) Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci 13(8):3266-3283.

Supplementary Material

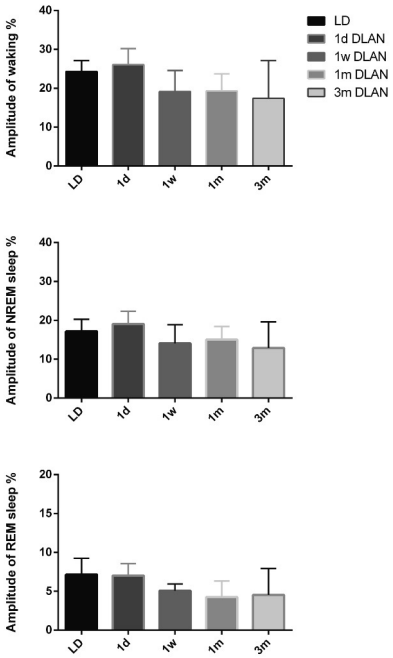

Figure 4.1: Amplitude of the daily rhythm of vigilance states (Waking, NREM and REM sleep) for control (LD), 1 day, 1 week, 1 month (n=5) and 3 months (n=9) dim-light-at-night (DLAN) conditions during 24-h baseline recordings (Mean $\pm$ SD).

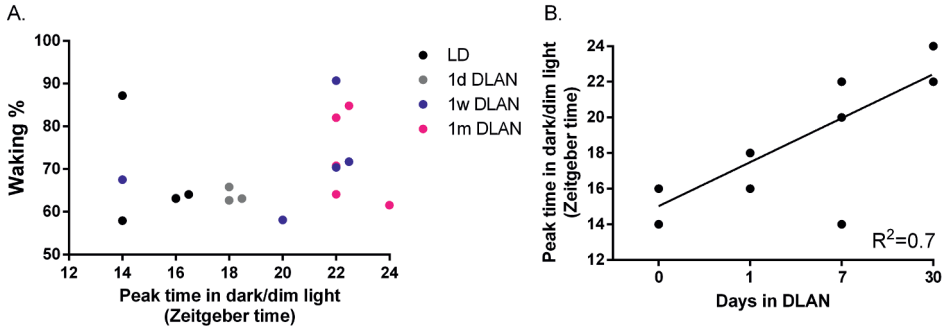

Figure 4.2: Relationship between the waking peak time during the dark/dim-light period (active period) and the amount of time spent in dim-light-at-night (DLAN). A. The amount of waking during the 2-h peak waking time for the experimental groups (control LD, 1 day DLAN, 1 week DLAN, 1 month DLAN). B. A positive correlation was found between the waking peak time and the amount of time spent in the DLAN condition from 0 days up to 30 days (n=5 for each condition) ($R^2 = 0.6999$).

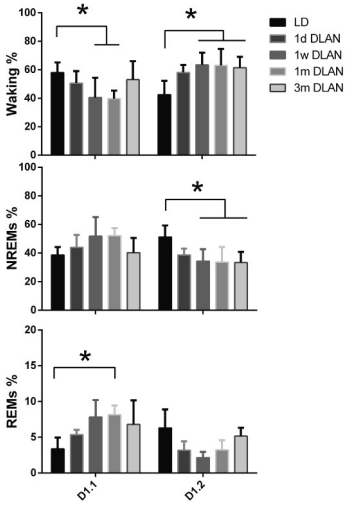

Figure 4.3: Distribution of each behavioral state (Waking, NREM and REM sleep) during the baseline dark/dim light period. Bar plots represent mean (± SD) values (D1.1, D1.2) corresponding to 6-h values for dark/dim light periods for control (LD), 1 day, 1 week, 1 month (n=5) and 3 months (n=9) dim-light-at-night (DLAN) conditions. Asterisks indicate significant differences between the groups (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, p<0.05 after significant ANOVA, main effects 'treatment', 'Light-Dark').

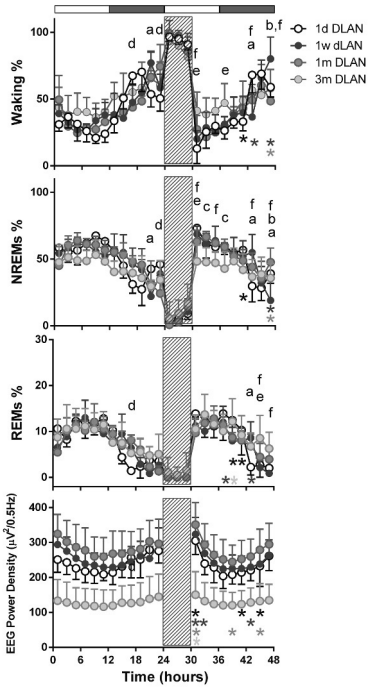

Figure 4.4: Time course of vigilance states and EEG power density in NREM sleep in 0.5-4.0 Hz (EEG SWA), for 24-h baseline (BL), 6-h sleep deprivation (hatched bar) and 18-h recovery for 1 day, 1 week, 1 month (n=5) and 3 months (n=9) dim-light-at-night (DLAN) conditions. Curves connect mean (± SD) 2-h values of Waking, NREM and REM sleep. The white and grey bars above each graph indicate the light-dim light cycle (inactive-active period respectively). Letters at the top of each graph represent significant differences between the groups (a: 1d-1w, b: 1w-1m, c: 1m-3m, d: 1d-1m, e: 1d-3m, f: 1w-3m DLAN) across the 48-h period and asterisks at the bottom of each graph significant differences between recovery and BL day for each DLAN group (post-hoc unpaired and paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVAs, main effects 'treatment', 'time of day', 'day'). Characteristic low EEG SWA levels were found in the three months DLAN group compared to all other groups.