Sleep alterations in the course of aging environmental inputs

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Chapter 8

Sleep and chronic dim-light-at-night in aging

Effects of chronic dim-light-at-night exposure on sleep in young and aged mice

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Abstract

Dim-light-at-night (DLAN) exposure is associated with health problems, such as metabolic disruptions, immunological modulations, oxidative stress, sleep problems, and altered circadian timing. Neurophysiological parameters, including sleep patterns, are altered in the course of aging in a similar way. Here, we investigated the effect of chronic (three months) DLAN exposure (12L:12Dim-light, 75:5lux) on sleep and the sleep electroencephalogram (EEG), and rest-activity behavior in young (6-month-old, n=9) and aged (18- n=8, 24-month-old, n=6) C57BL/6J mice and compared with age-matched controls (n=11, n=9 and n=8, respectively). We recorded the EEG and electromyogram continuously for 48-h and conducted a 6-h sleep-deprivation. A delay in the phase angle of entrainment of locomotor activity and daily vigilance state rhythms was apparent in mice following DLAN exposure, throughout the whole age spectrum, rendering sleep characteristics similar among the three age DLAN groups and significantly different from the age-matched controls. Notably, slow-wave-activity in NREM sleep (SWA, EEG power density in 0.5-4.0 Hz) was differentially altered in young and aged DLAN mice. Particularly, SWA increased as a function of age, which was further accentuated following DLAN exposure. However, this was not found in the young DLAN animals, which were characterized by the lowest SWA levels. Concluding, long-term DLAN exposure induced more pronounced alterations in the sleep architecture of young mice, towards an aging phenotype, while it enhanced age-associated sleep changes in the older groups. Our data suggest that irrespective of age, chronic DLAN exposure deteriorates sleep behavior and may consequently impact general health.

Keywords: sleep, dim-light-at-night (DLAN), aging, electroencephalogram, sleep deprivation, slow-wave-activity

Introduction

The invention of electrical light in the past century has entirely reformed modern society. Although generally it is considered an everyday convenience, artificial light exposure particularly at night, has been linked with health problems such as metabolic, immunological, and behavioral rhythm disturbances across a wide age spectrum [1]. One such rhythm vulnerable to perturbation, is the sleep-wake cycle [2, 3, 4].

Sleep is mainly regulated by two processes, a circadian process, which is controlled 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 [6, 7]. 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) [6, 7]. Sleep deprivation has been experimentally used to test the sleep homeostatic process and investigate sleep characteristics such as sleep architecture and the sleep EEG under elevated sleep pressure conditions [7].

Concomitant to a multitude of physiological parameters that are affected owing to aging, sleep does not remain unaltered. Aging in laboratory animals, such as mice, is characterized by increased NREM sleep during the dark active period, emerging from an increase in longer NREM sleep episodes, as well as increased EEG SWA levels [8, 9, 10, 11, 12, 13, 14]. In addition to increased EEG SWA, the overall morphology of slow-waves has been found to be modulated, likely indicating changes in connectivity of cortical brain areas in the course of aging [8, 9].

The combination of aging and long-term light-at-night exposure may have additional effects on sleep and the sleep EEG. Whole-night light exposure (40 lux) in young human subjects shows acute effects on sleep architecture and brain activity [15]. In particular, shallow sleep, frequent arousals, reduction of activity in the slow-wave and spindle frequency bands during NREM sleep and theta frequency bands during REM sleep have been found. In a similar group of subjects, dim-light-at-night (DLAN) exposure (5 lux) has been shown to affect sleep quality, increasing waking after sleep onset, NREM sleep stage 1 and REM sleep, whereas decreasing NREM sleep stage 2 [16]. In animals, disruption of molecular circadian rhythms and weight gain in mice [17], disturbance of the daily sleep-wake cycle in the rat [18], as well as altered daily rhythms and behavioral patterns in the grey mouse lemur [19] have been demonstrated following DLAN exposure. Although detrimental effects of DLAN exposure, particularly in the long-term, have been suggested, the impact in aging on sleep is not documented yet and is important as both the aging population and light exposure have increased in the last decades.

In the current study, the effect of long-term DLAN exposure (5 lux) on behavior, sleep architecture and the sleep EEG in aged mice is investigated. 18- and 24-month old mice are exposed to three consecutive months of DLAN and the effects are compared to young 6-month old mice exposed to the same treatment as well as to age-matched controls exposed to a light-dark cycle. A delay in the phase angle of entrainment of daily vigilance state rhythms was noted in mice across the whole age spectrum and sleep characteristics between young and aged mice shared many similarities following DLAN exposure. Additionally, sleep changes already seen in aged mice, such as the attenuated 24-h vigilance state rhythm amplitude, the increase in NREM sleep particularly during the dark period, as well as elevated SWA levels, were further augmented in aged mice after DLAN expo-

sure. The data indicate that although the most pronounced effects are found at a young age, chronic DLAN exposure is detrimental as far as the sleep process is concerned, irrespective of age.

Materials and methods

Animals

Male C57BL/6JOlaHsd mice of three age groups (6, 18 and 24 months old, n=51) (Harlan, Horst, the Netherlands) were used for the current study. All mice were exposed to light:dark conditions [(12:12 h, 75lux:0lux), with 0 lux corresponding to complete darkness (1lux=1 lm/m2)]. Three months before the surgeries mice were partitioned into two groups [at the age of 3 months for the 6 months old group (n=11 and n=9 for control and DLAN exposed), at the age of 15 months for the 18 months old group (n=8 and n=8 for control and DLAN exposed) and at the age of 21 months for the 24 months old group (n=9 and n=6 for control and DLAN exposed)]. Mice were individually housed under controlled conditions [12:12 h light:dark cycle (75lux:0lux) (Control group) or 12:12 h light:DLAN (75lux:5lux) (DLAN group); lights on at 10:00AM] with food and water ad libitum in a temperature controlled room (21-22 °C). Light in all conditions was emitted by white fluorescent tubes placed above the cage. Light intensities were verified by AvaSpec 2048-SPU (Avantes BV, the Netherlands) light meter.
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 [9, 21]. The wire branches of all electrodes were set in a plastic pedestal (Plastics One, Roanoke, VA) which was fixed to the skull with dental cement. The mice were allowed to recover for 7-10 days.

Light schedules and behavioral recordings

The mice were kept on a 12:12 h Light:Dark schedule. In order to investigate the effect of long-term DLAN exposure on behavior, vigilance states and the EEG, the mice were exposed for 12 weeks to 12:12h Light:DLAN. Subsequently EEG and EMG electrodes were implanted and sleep recordings were performed. Locomotor activity for the light-dark schedule (15 days) and the three-month DLAN condition was monitored by passive infrared detectors (PIR, Actimetrics software, Wilmette, Il, USA) prior to the EEG/EMG recordings. The rhythm period and strength of the 24-h component was determined by F-Periodogram analysis, a method generally designed to detect rhythmic components in rest-activity data, similar to previous studies [18, 20]. The strength of the 24-h component particularly is defined as the difference between the peak and the 99% confidence

limit in individual periodograms of rest-activity data, an approach elaborately described earlier to quantify the magnitude of the 24-h component [20].

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 [9, 21]. Before each recording, a calibration signal (10 Hz sine wave 300 μV peak-to-peak) 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 baseline (BL, 24h) day was recorded, starting at lights on. Subsequently, a second day was recorded at the start of which, six hours of sleep-deprivation were conducted by gentle handling [9, 21, 22]. EEG and EMG were recorded continuously during BL and sleep deprivation, as well as for 18 hours of recovery period. In total, continuous 48h were recorded including the BL, the sleep deprivation, and 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 described previously [9, 21, 22]. For each epoch, the EEG power density in the slow-wave (delta activity, 0.75-4.0 Hz) and faster frequency range (theta activity, 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. In general, we tested for and found distributions and equal variances in the sampled distributions and therefore parametric tests were used. To test whether DLAN or age altered the daily vigilance state distribution, a one-way ANOVA was performed on 24-h mean values (factor 'treatment', 'age') and three-way ANOVA was performed on 12-h and 6-h and 2-h mean values of vigilance states (factors 'treatment', 'age', 'Light-Dark', 'time of day', 'day'). Note that L2 corresponds to 6-h value of light period following sleep deprivation and is compared to the corresponding 6-h value of BL. Additionally, to test potential differences in SWA in NREM sleep, a one-way with factor 'group' and a three-way ANOVA with factors 'treatment', 'time of day', 'day' for all groups was performed, as well as a two-way ANOVA with factors 'time of day' and 'day' for each group to test potential alterations due to sleep deprivation. A two-way ANOVA on the 24-h EEG power density values of the three vigilance states with main factors 'treatment' and 'EEG frequency bins' was performed. Cumulative NREM and REM sleep plots were calculated by subtracting the minutes of sleep during deprivation and recovery from the corresponding baseline value

and summing the difference with the preceding hour and were tested through a two-way ANOVA with factors 'treatment', 'age' and 'time of day'. Additionally, linear regressions of the accumulated NREM sleep and REM sleep regained during the 18-h recovery period were computed and slope differences of the linear regression were tested with unpaired t-tests. To test the strength of the behavioral rhythm, a one-way ANOVA was performed with factor 'strength' across 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

Examples of representative rest-activity behavior from animals exposed to LD and DLAN conditions of the three different age groups are shown in Figure 1 (15 days). By performing F-periodogram analysis, we computed the rhythm period which revealed one peak in all mice not significantly different from 24 hours (Mean±SD, Young LD: 24 $\pm$ 0.0, Young DLAN: 24.08 $\pm$ 0.21, 18m LD: 24.02 $\pm$ 0.05, 18m DLAN: 23.97 $\pm$ 0.25, 24m LD: 24.01 $\pm$ 0.072, 24m DLAN: 24.51 $\pm$ 0.48). In addition, the strength of the behavioral rhythm was computed (Mean$\pm$SD, Young LD: 0.21 $\pm$ 0.02, Young DLAN: 0.11 $\pm$ 0.03, 18m LD: 0.12 $\pm$ 0.03, 18m DLAN: 0.09 $\pm$ 0.03, 24m LD: 0.15 $\pm$ 0.03, 24m DLAN: 0.12 $\pm$ 0.04) and was found decreased in all DLAN mice as compared to age-matched controls with significant results in young and 18 months old mice ($p<0.05$) as well as in both 18 and 24 months old control groups compared to the young control group but across DLAN mice (One-way ANOVA, factor 'strength' $p=0.0001$, post-hoc unpaired t-tests between groups).

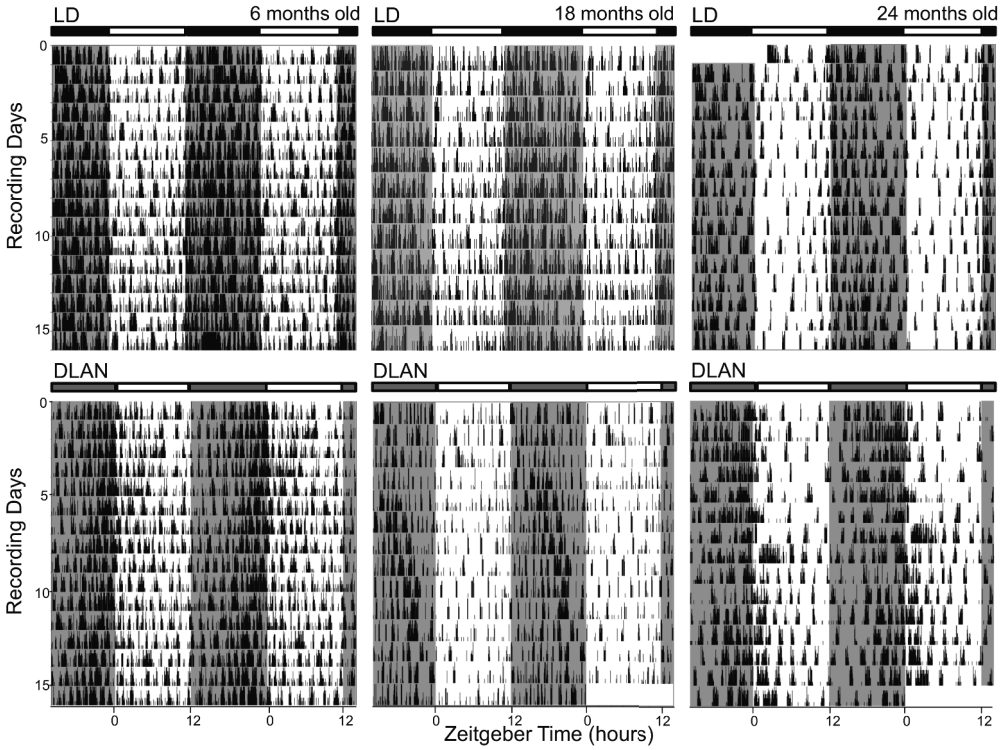

Figure 8.1: Representative double-plotted actograms of 3 mice of three different age groups (6, 18, 24 months old) exposed to control 12:12h light:dark (top, LD, 75lux:0lux) (15 days) and 12:12h light:dim-light-at-night (bottom, DLAN, 75lux:5lux) (15 days). Note the concentration of activity to the later part of the dim period in the DLAN group.

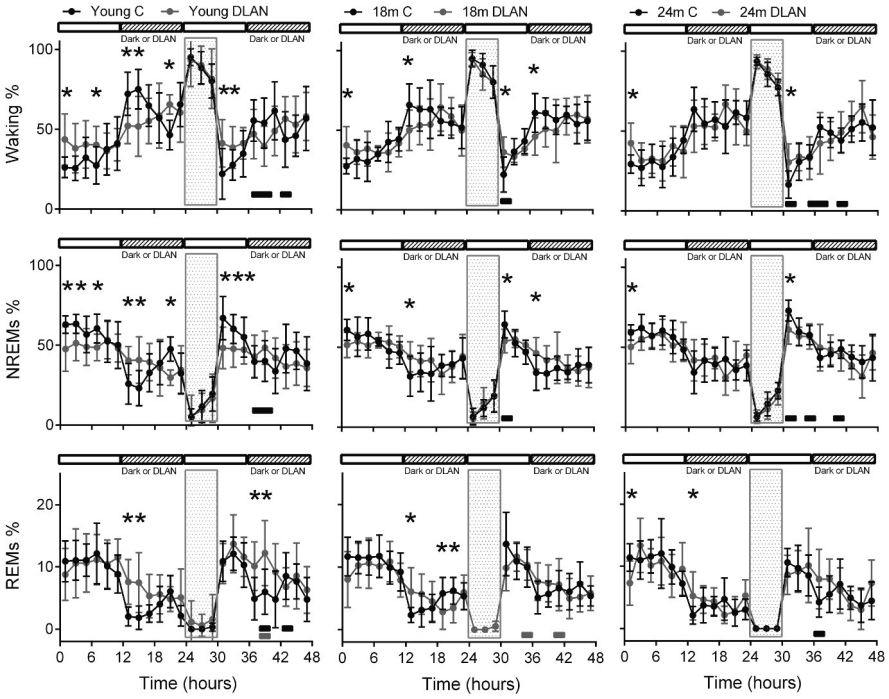

Figure 8.2: Time course of vigilance states, for 24-h baseline, 6-h sleep-deprivation (hatched bar) and 18-h recovery period for young, 18 and 24 months old mice in either control light:dark conditions (n=11, 8, 9 respectively) or following 3 months exposure to dim-light-at-night (DLAN) conditions (n=9, 8, 6 respectively). Curves connect 2-h values of waking, NREM and REM sleep (Mean $\pm$ SD). The bars above each graph indicate the light-dark/dim light cycle. Black asterisks at the top of each graph represent significant differences between the control and the DLAN mice for each age group across the 48-h period, while bars at the bottom represent differences between baseline day and recovery day (post-hoc unpaired and paired t-tests with Bonferroni multiple comparisons correction when appropriate, $p<0.05$ after significant ANOVAs, main effects 'treatment', 'time of day', 'day').

Vigilance states: Waking, NREM and REM sleep amount

The largest differences regarding the effects of DLAN on the amount of vigilance states were found in the young DLAN mice compared to age-matched controls. In particular, increased wakefulness was found in young DLAN in the light period and decreased wakefulness during the dim light/dark period, as well as more REM sleep in the dim light/dark period (Figure 2) (post-hoc unpaired t-tests Waking: $F(11,432)=1.5$; $p=0.13$, NREM: $F(11,432)=1.63$; $p=0.088$ and REM sleep: $F(11,432)=0.69$; $p=0.747$ with significant interactions 'treatment*time of day' $p<0.0001$, Appendix, Table A.1), with more REM sleep apparent in the 12-h dim light/dark period and more waking in the light period following sleep deprivation (Figure 3) (post-hoc unpaired t-tests Waking: $F(1,72) = 0.0001$; $p=0.986$, NREM sleep: $F(1,72) = 0.034$; $p=0.855$ and REM sleep: $F(1,72) =$

0.353; p=0.554 with significant interactions 'treatment* Light-Dark' p<0.05, Appendix, Table A.1). Notably, all 6, 18 and 24 months old DLAN mice showed a delay in the increase in waking at the beginning of the dim light period and a similar delay in the increase in NREM sleep at the start of the light period, compared to age-matched controls (post-hoc unpaired t-tests, 18 months old, Waking: F(11,336)=0.954; p=0.489, NREM sleep: F(11,336)=0.859; p=0.581 and REM sleep: F(11,336)=0.672; p=0.765 and 24 months old, Waking: F(11,306)=0.84; p=0.6, NREM sleep: F(11,306)=0.894; p=0.547 and REM sleep: F(11,306)=0.642; p=0.792 with significant interactions 'treatment*time of day' p<0.05, Appendix, Table A.1) inducing in turn an attenuated 24-h vigilance state rhythm amplitude (defined as the vigilance state difference between light and dark periods). These effects were not only apparent during BL, but were also consistent in the recovery period following sleep deprivation. Additionally, compared to BL, sleep deprivation did not induce any alterations in the amounts of waking and NREM sleep in all DLAN mice, contrary to changes induced in the age-matched control mice; a subtle increase was only evident in REM sleep in the dim light period after sleep deprivation in young and 18 months old DLAN mice compared to BL (post-hoc paired t-tests between BL and after sleep deprivation period values, following significant interactions 'treatment*time of day' and main factor 'day' p<0.05, Appendix, Table A.1).

24-h and 12-h values did not differ in 18 and 24 months old mice long-term exposed to DLAN compared to age-matched controls (p>0.05) (Figure 3). Compared to BL, young, 18 and 24 months old DLAN mice had more REM sleep in the dim light period following sleep deprivation (post-hoc paired t-tests between BL and after sleep deprivation period values, following significant interactions 'treatment*Light-Dark' and main factor 'day' p<0.05, Appendix, Table A.1). In contrast, young, 18 and 24 months old control animals showed more alterations in their vigilance state distribution after sleep deprivation, including more NREM and REM sleep and less waking in the dark period in the young mice, less waking and more NREM sleep in the dark period in 18 months old mice, and less waking and more NREM sleep in both light and dark periods in 24 months old mice (post-hoc paired t-tests, between BL and after sleep deprivation period values, following significant interactions 'treatment*Light-Dark' and main factor 'day' p<0.05, Appendix, Table A.1).

Mice exposed to long-term DLAN showed a gradual increase as a function of age in NREM sleep, particularly evident in the 6-h light period after sleep deprivation as well as decreased REM sleep in the 12-h dim light period after sleep deprivation, with significant differences between young and 24 months old mice (Figure 4) (post-hoc unpaired t-tests, NREM sleep: F(2,88)=0.6; p=0.55, with significant interaction 'age*Light-Dark' and main factors p<0.05, REM sleep: F(2,88)=0.593; p=0.555 with main factors 'Light-Dark' and 'day' p<0.05, Appendix, Table A.1). 24-h BL values did not differ between the young mice long-term exposed to DLAN and both 18 and 24 months old (p>0.05). Interestingly, waking, NREM and REM sleep 2-h values did not differ among the three DLAN age groups (p>0.05) in the BL day, with only small decreases in REM sleep during the second day following sleep deprivation, in the oldest age groups compared to the young (Figure 5) (post-hoc unpaired and paired t-tests, F (22,480) = 0.366; p=0.997, with main factors 'treatment', 'time of day' and 'day' <0.0001, Appendix, Table A.1).

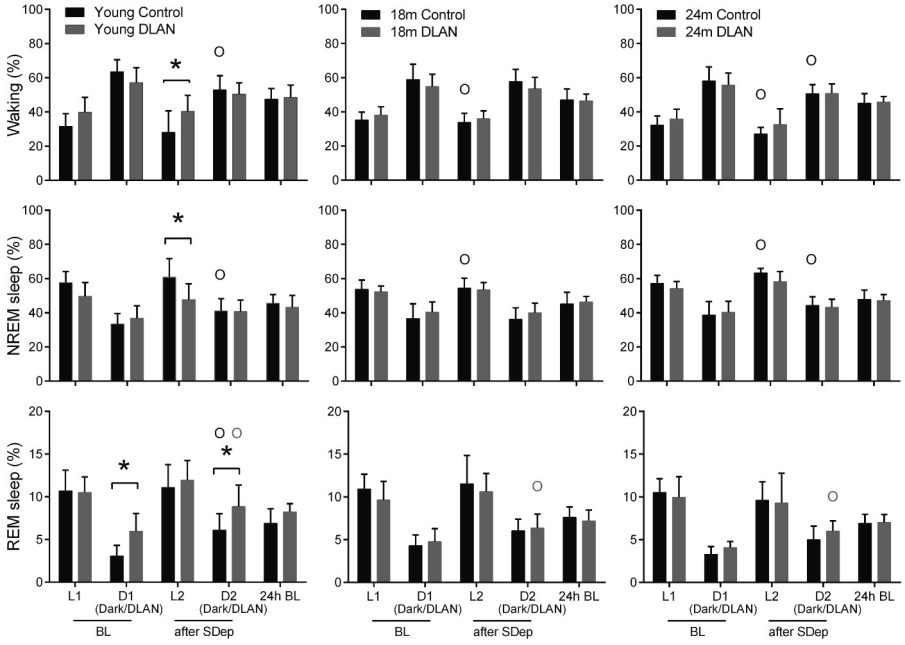

Figure 8.3: Distribution of each behavioral state (Waking, NREM and REM sleep) during the baseline day and after sleep deprivation. Bars represent mean ($\pm$ SD) values (L1, D1 (data during baseline (BL)), D2 correspond to 12-h values and L2 (L2,D2: data after sleep deprivation) to 6-h values for the 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 the three age groups control light:dark conditions (n=11, 8, 9 for young, 18 and 24 months old) or following three months exposure to dim-light-at-night (DLAN) conditions (n=9, 8, 6 respectively). Asterisks indicate significant differences between the groups and circles indicate significant differences between recovery and baseline day (post-hoc unpaired and paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVAs, main effects 'treatment', 'Light-Dark', 'day').

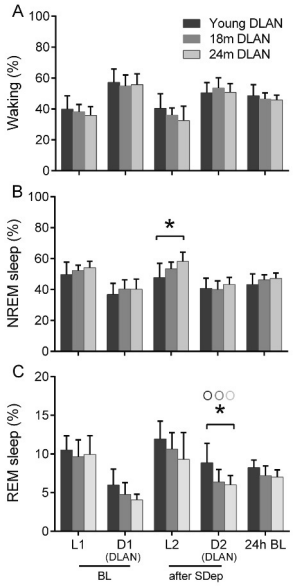

Figure 8.4: Distribution of each behavioral state (Waking, NREM and REM sleep) during the baseline day and after sleep deprivation in the age groups long-term exposed to dim-light-at-night (DLAN). Bars represent mean (± SD) values (L1, D1 (data during baseline (BL)), D2 correspond to 12-h values and L2 (L2, D2: data after sleep deprivation) 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 (A), NREM sleep (B) and REM sleep (C) for the three age DLAN groups (young: n=9; 18 and 24 months old: n=8 and 6). D1 and D2 correspond to dim light periods. Asterisks indicate significant differences between the age groups and circles indicate significant differences between recovery and baseline day for the same group, shown in gradually decreasing gray colors (post-hoc unpaired and paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'age', 'Light-Dark', 'day').

Additionally, we computed the cumulative NREM and REM sleep lost or gained (Figure 6) (post-hoc unpaired t-tests, $F(46,480)=0.34$; $p>0.99$ with main factors 'age' $p<0.0001$, 'time of day' $p<0.0001$). Although a significant part of NREM sleep lost was not regained in all groups, DLAN or aging and their combination led to less NREM sleep regained, as compared to young control mice ($p<0.05$). The slopes of the regained NREM sleep differed significantly between young control and young DLAN mice (Rate of accumulated NREMs percentage/hour, young control: 7.908 ± 1.127; young DLAN: 2.816 ± 1.553; $p=0.007$) and between the three age control groups (young control: 7.908 ± 1.127; 18 months control: 0.5108 ± 1.335; 24 months control: 3.843 ± 1.155; $p<0.0001$). Interestingly, young DLAN mice showed similar patterns to aged DLAN mice ($p>0.05$).
Contrary to NREM sleep, REM sleep lost was almost fully regained in young DLAN mice showing an increase during recovery similar to young controls, whereas aged con-

trol and DLAN mice were not able to regain the lost REM sleep showing a less steep increase (post-hoc unpaired t-tests, Appendix, Table A.1) with slopes significantly different between age control groups (young control: 2.78 $\pm$ 0.29; 18 months control: 1.819 $\pm$ 0.32; 24 months control: 1.397 $\pm$ 0.22; p=0.0012) a trend between age DLAN groups (young DLAN: 2.752 $\pm$ 0.43; 18 months DLAN: 1.853 $\pm$ 0.32; 24 months DLAN: 1.66 $\pm$ 0.27; p=0.07576).

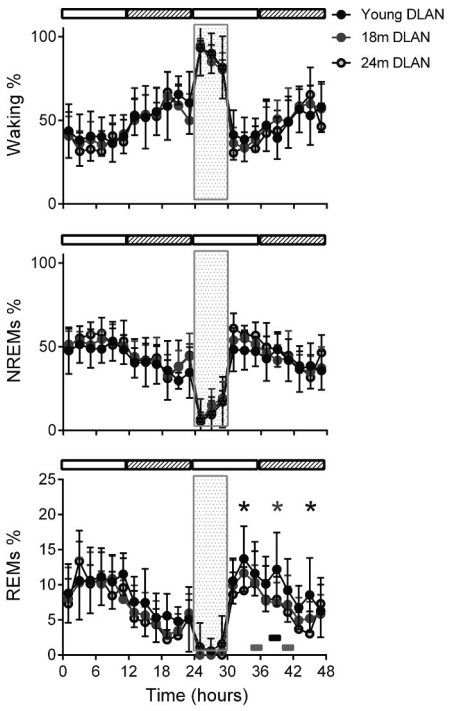

Figure 8.5: Time course of vigilance states, for 24-h baseline, 6-h sleep-deprivation (hatched bar) and 18-h recovery period for young, 18 and 24 months old mice following 3 months exposure to dim-light-at-night (DLAN) conditions (n=9, 8, 6 respectively). Curves connect 2-h values of waking, NREM and REM sleep (Mean $\pm$ SD). The bars above each graph indicate the light-dim light cycle. The gray and black asterisks indicate significant differences between the young and the 18 and 24 months old mice respectively (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction when appropriate, p<0.05 after significant ANOVA, main effects 'treatment', 'time of day', 'day').

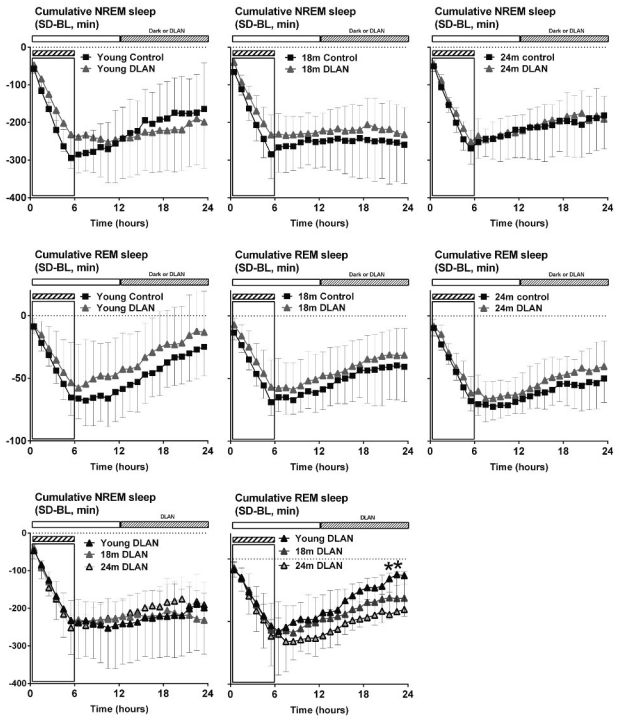

Figure 8.6: Time course of accumulated NREM sleep and REM sleep lost and regained during the 6-h sleep deprivation and the 18-h recovery period for young, 18 and 24 months old mice in either control light:dark (n=11, 8, 9 respectively) or following three months exposure to light:dim-light-at-night (DLAN) conditions (n=9, 8, 6 respectively). Curves connect 1-h values that are calculated by subtracting the minutes of sleep during deprivation and recovery from the corresponding baseline value and summing the difference with the preceding hour. Asterisks indicate significant differences between young DLAN and 24 months DLAN mice (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment'/'age', 'time of day'). The slopes of the regained NREM sleep differed significantly between young control and young DLAN mice and between the three age control groups (*more details in the Results section of the text*).

EEG power density between 0.5-25 Hz and slow-wave activity in NREM sleep

The most pronounced differences regarding the EEG power spectra were found in the young mice groups (Figure 7). Young DLAN mice showed a decrease in the slow frequencies in the waking (1-5 Hz), and NREM sleep (1-4.5 Hz) spectrum (post-hoc unpaired t-tests, Waking: $F(29,540)=4.9$; $p<0.0001$, NREM sleep: $F(29,540)=5.93$; $p<0.0001$, Appendix, Table A.1). In the REM sleep spectrum, DLAN exposure decreased power in the slow wave and theta frequencies (3-3.5 and 8 Hz) (post-hoc unpaired t-tests, REM sleep: $F(29,540)=1.348$; $p=0.1082$ with main factors 'treatment' and 'EEG frequency bins' $p<0.0001$, Appendix, Table A.1). In contrast, DLAN exposure did not induce any alterations in the waking, NREM and REM sleep spectra of the 18 months old mice

($p>0.05$). In the 24 months old mice, however, long-term DLAN exposure induced alterations in the opposite direction compared to the young mice, with increased activity in the slow waves in waking and NREM sleep (0.5 and 3.5 Hz in waking, and 2.5-3.5 in NREM sleep), as well as increased theta activity during REM sleep (7-8 Hz) (post-hoc unpaired t-tests, Waking: $F(29,390)=1.5$; $p=0.0557$, NREM sleep: $F(29,390)=2.2$; $p=0.0006$, REM sleep: $F(29,390)=1.15$; $p=0.2786$, with main factors 'treatment' and 'EEG frequency bins' $p<0.0001$, Appendix, Table A.1).

SWA in NREM sleep was differentially affected in young and aged DLAN mice as compared to their age-matched controls. A characteristic increase in SWA was apparent as a function of age, as previously observed [8, 9]. Chronic DLAN exposure induced an overall further increase in the 24 months old mice in the levels of SWA across the 48-h recording time (Figure 8) ($F(55,1033)=0.093$; $p>0.99$, with main factors 'treatment' $p<0.0001$ 'time of day' $p=0.057$, 'day' $p=0.006$, Appendix, Table A.1). In contrast, a decrease in the levels of SWA across the 48-h was found following chronic DLAN exposure in the young mice. SWA in NREM sleep was significantly different across the 48-h among groups, except for the 18 months old control, 18 months old DLAN and 24 months old control mice which shared similar values (One-way ANOVA, factor 'group', $p<0.0001$; post-hoc t-tests between groups in the overall period, $p<0.05$). Following sleep deprivation, an increase in SWA was evident in all groups (post-hoc paired t-tests, Appendix, Table A.1). This increase lasted between 2-6 h in the light period following sleep deprivation.

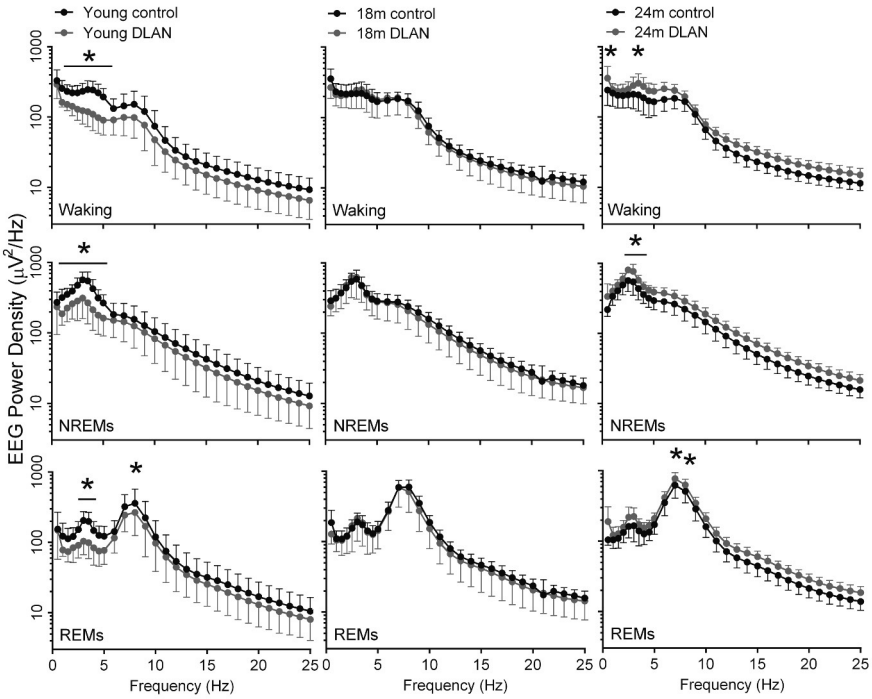

Figure 8.7: EEG power density in Waking, NREM and REM sleep for young, 18 and 24 months old mice in either control light:dark (black circles, n=11, 8, 9 respectively) or following three months exposure to light:dim-light-at-night (DLAN) conditions (gray circles, n=9, 8, 6 respectively) in the frequencies between 0.5 and 25 Hz computed for pooled values of the 24-h baseline day. Between 0.25 and 5.0 Hz, values were calculated in 0.5-Hz bins and between 5.25 and 25.0 Hz in 1-Hz bins. Black asterisks indicate significant differences between the control and the DLAN mice of each age group (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment', 'EEG frequency bin').

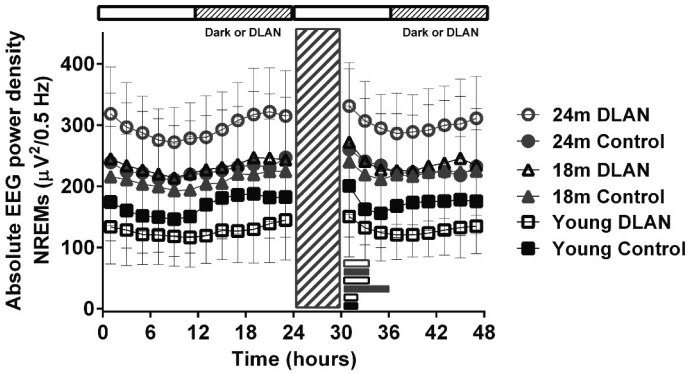

Figure 8.8: Time course of EEG slow-wave activity in NREM sleep (SWA, EEG power density between 0.5-4.0 Hz) for 24-h baseline, and 18-h recovery period following sleep deprivation (hatched bar) for young, 18 and 24 months old mice in either control light:dark conditions (n=11, 8, 9 respectively) or following 3 months exposure to dim-light-at-night (DLAN) conditions (n=9, 8, 6 respectively). The bars above each graph indicate the light-dark/dim light cycle. EEG SWA increases as a function of age and following long-term DLAN exposure it increases further in aged mice, while it decreases in young mice. Bars at the bottom (in line with the group legends colors) of the plot indicate differences between baseline day and recovery day for each group (post-hoc paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVAs, main effects 'treatment', 'time of day', 'day').

Discussion

In the current study, we show that three continuous months of DLAN exposure has detrimental effects on sleep architecture, the sleep EEG, and rest-activity behavior across a large age spectrum. Regarding the sleep architecture, a characteristic delay of the 24-h vigilance state rhythm was found in all DLAN mice as compared to age-matched controls. An aged sleep phenotype was found in young mice exposed to long-term DLAN, and additional age alterations were apparent in aged DLAN mice. Interestingly, the factors DLAN, aging as well as their combination led to a decrement in recovery of the amount of lost NREM sleep following sleep deprivation. In contrast to the effect of DLAN on recovery of NREM sleep in young mice, REM sleep was almost fully regained in young mice exposed to DLAN. Notably, SWA in NREM sleep was differentially altered in young and aged mice. It increased as a function of age, which was further accentuated following three months of DLAN exposure in the 24 months old mice but not in the 18 months old. However, the lowest SWA levels among all groups were found in young DLAN mice. The data demonstrate, for the first time, that long-term DLAN exposure induces an aged behavioral sleep phenotype in young mice, and accentuates age-associated sleep changes in aged mice.

Sleep and behavior

Aging phenotype and circadian disruption

Pronounced sleep alterations following a long-term DLAN exposure, were found in

young mice as compared to age-matched controls. More waking and less NREM sleep were found in the light period, while the opposite was apparent in the beginning of the dim light period along with more REM sleep. Additionally, investigating the vigilance states across the 48-h among the three age groups, a similar characteristic pattern was evident in the young, 18 and 24 month old DLAN mice, showing a similar decrease in the daily amplitude of vigilance state rhythms following chronic DLAN exposure irrespective of age (Figure 5). A recent study in Wistar rats found a gradual reduction in rhythm amplitude in vigilance state distribution, starting from day 1 to day 14 of DLAN exposure [18]. Aged mice, as shown earlier, are characterized by sleep changes such as more NREM sleep and less waking during the dark/active period as well as less REM sleep at the end of the light/inactive period [10, 11, 12, 13, 14, 9, 8]. Our data demonstrate that, following chronic exposure to DLAN, behavioral sleep features converge to an aged sleep phenotype.

Behavioral data in our study demonstrate that in the course of aging the amount of locomotor activity decreases. Moreover, with the introduction and chronic exposure to DLAN, a delay in the phase angle of entrainment is noted in the 24-h rest-activity rhythm across all ages, which is also evident in the delay of each vigilance state in the 2-h sleep-wake values (Fig.1 and Fig. 2). Delayed temperature rhythms were also found after only 2 weeks of DLAN exposure in the grey mouse lemur [19]. In addition to delayed rhythms, the rhythm strength is also found attenuated following DLAN exposure in all groups, suggesting that DLAN affects the circadian clock and particularly the magnitude of the 24-h component [18, 20]. Notably, DLAN adds more effects in the aged circadian clock, decreasing further the strength of the circadian rhythm.

Although mice and humans obtain different characteristics with reponse to light, with the former being nocturnal and the latter being diurnal, our study has translational value regarding sleep and circadian disturbances owing to DLAN. DLAN exposure at the dark period in mice can affect the circadian clock and induce sleep alterations that impact not only the active, but, as a consequence the inactive period too. Generally, the rodent and human circadian timing system share many common features. In particular, the SCN rhythm of firing activity and neuropeptide expression is largely similar between nocturnal and diurnal mammalian species [24]. The present study suggests long-term DLAN in humans may influence their circadian clock in a similar way to mice, showing rhythm disturbances and affecting consequently sleep architecture. Future studies in diurnal animals may validate our hypotheses.

We found in our study that the NREM sleep lost during sleep deprivation was not fully regained in either of the experimental groups. In general, if a sleep homeostatic response occurs, a complete recovery of the amount of sleep is not necessary as sleep will be intensified, with high SWA during recovery [25]. However, young mice, chronically exposed to DLAN, showed an attenuated response to sleep deprivation compared to age matched controls and recovery sleep in this group resembled that of the aged mice exposed to DLAN. Aging can modify the response to sleep deprivation on NREM sleep amount and the pattern of recovery sleep duration [11]. As we show in our results, the homeostatic response declines in aging, and when alterations in light, such as DLAN, are added, this response is further reduced. In contrast to NREM sleep lost, REM sleep lost during the sleep deprivation was fully recovered in the young mice, even following chronic DLAN exposure. This is in contrast to the aged mice, as also reported earlier [11]. There-

fore, the mechanism of REM sleep homeostatic regulation may differ from NREM sleep regulation, as the former is not affected by DLAN exposure in young mice.

The sleep EEG: Accentuated age-induced characteristics

In addition to alterations found in young mice in slow-wave and theta frequencies in the waking, NREM and REM sleep spectra, which could reflect a general degradation in connectivity in EEG generating brain areas, as for instance the cortex and the hippocampus, it is evident in the present study that long-term DLAN exposure affects the sleep EEG in the course of aging. Compared to control, 18 months old mice did not show any changes in the EEG spectra when exposed to DLAN, but 24 months old mice demonstrated an increase in activity in the slow frequencies of waking and NREM sleep spectra as well as in theta frequencies in the REM sleep spectrum. This is in contrast with the findings in young mice, which are characterized by a general decrease in power following chronic DLAN exposure. Aged DLAN mice likely show increased drowsiness as indicated by the increase in activity in the slow-waves in the waking spectra [26, 27]. In a parallel way, a further increase in slow-waves in NREM sleep suggests that aging and DLAN exposure act synergistically. In particular, it is known that aged mice show an increased power in the slow-wave range of the NREM sleep EEG [8, 9]. Taking into consideration that following moderate physical activity, being considered a healthy intervention that can ameliorate several body and brain characteristics, EEG SWA decreases in aged mice [5], while it increases following long-term high-caloric diet in aged mice [28], it can be suggested that DLAN accentuates aging characteristics, as EEG SWA increases in 24 month old DLAN mice.

Concerning the latter assumptions, the question arises whether the SWA decrease found in the young mice implies a beneficial or detrimental effect of DLAN on the sleep EEG (Figure 8). Although it has been demonstrated that following the healthy intervention of running wheel, EEG SWA is decreased in an analogous way in both young and aged mice [5], here, the decrease in EEG SWA in young mice following long-term DLAN exposure, stems from the general attenuation in EEG power in all the prominent frequencies, including theta activity during waking and REM sleep, and therefore it is probably not an indication of improved health, but probably of a decrease in brain network integrity. We have suggested previously that aged mice have altered brain network properties compared to young animals, with overall reduced plasticity and possibly increased network connectivity in local circuits and weaker synchronization at the global level [9], resulting in higher SWA during NREM sleep. Additionally, all the EEG SWA responses to environmental changes, such as a high caloric diet or increased physical activity [5, 28], is less robust in aged compared to young animals. Accordingly, following chronic DLAN exposure, the cortical network at the local level is possibly only modestly affected in the aged mice, showing simply an increase in brain aging phenotype with more SWA in the EEG seen in the 24 months old mice, since generally the local cortical neural dynamics are not impaired during healthy senescence [8]. In contrast, the brain network of young mice, highly plastic and globally synchronized, is more profoundly affected at many levels, with a complicated response as an output that indicates overall an enhanced susceptibility to external inputs.

In conclusion, our data indicate that deleterious effects on sleep prevail following long-

term DLAN exposure across all ages. A diminished amplitude of the 24-h vigilance state rhythm is apparent in young mice exposed to DLAN, similar to aged mice. Additionally, disrupted circadian rhythms are found with a characteristic delay of activity in all age groups. Notably, aging in combination with long-term DLAN exposure augmented the age-associated sleep characteristics. Particularly with regards to the sleep EEG, EEG SWA was found to be further increased in 24 months old mice, and the response to sleep deprivation was attenuated in both 18 and 24 months old mice. A different effect on sleep EEG was evident in young mice compared to the old; this consisted of a general EEG power attenuation in the prominent frequencies in all vigilance states as well as in the decreased EEG SWA pattern compared to all groups. In accordance with earlier studies investigating the effects of DLAN on general health, we demonstrate that chronic DLAN exposure disturbs the circadian clock and alters sleep and EEG characteristics across a large age span.

Competing interests

Authors declare no conflicts of interest.

Author contributions

T.DB. designed research; M.P. and T.DB. performed research; M.P. and T.DB. analyzed data; and M.P. and T.DB. wrote the paper.

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Table 8.1: Statistical analysis: two or three-way analysis of variance (ANOVA) was conducted to test the effect of age, treatment and sleep deprivation (see text for more details). Asterisks following reported p values indicate significance (* p<0.05).

Table A1. Detailed statistics			
Figure	Vigilance state	Two- or three-way ANOVA Interaction factors	Main factors
2	Waking (6 months old mice)	'treatment*day*time of day' F(11,432)=1.5 p=0.13 with 'treatment*time of day' p<0.0001*	'treatment' p=0.255 'day' p<0.0001* 'time of day' p<0.0001*
	NREM sleep (6 months old mice)	'treatment*day*time of day' F(11,432)=1.63 p=0.088 with 'treatment*time of day' p<0.0001*	'treatment' p=0.005 * 'day' p<0.0001* 'time of day' p<0.0001*
	REM sleep (6 months old mice)	'treatment*day*time of day' F(11,432)=0.69 p=0.747 with 'treatment*time of day' p<0.0001*	'treatment' p<0.0001* 'day' p=0.013 * 'time of day' p<0.0001*
2	Waking (18 months old mice)	'treatment*day*time of day' F(11,336)=0.954 p=0.489 with 'treatment*time of day' p<0.0001*	'treatment' p=0.219 'day' p<0.0001* 'time of day' p<0.0001*
	NREM sleep (18 months old mice)	'treatment*day*time of day' F(11,336)=0.859 p=0.581 with 'treatment*time of day' p=0.003 *	'treatment' p=0.099 'day' p<0.0001* 'time of day' p<0.0001*
	REM sleep (18 months old mice)	'treatment*day*time of day' F(11,336)=0.672 p=0.765 with 'treatment*time of day' p<0.0001*	'treatment' p=0.424 'day' p<0.0001* 'time of day' p<0.0001*
2	Waking (24 months old mice)	'treatment*day*time of day' F(11,306)=0.84 p=0.6 with 'treatment*time of day' p=0.011 *	'treatment' p=0.279 'day' p<0.0001* 'time of day' p<0.0001*
	NREM sleep (24 months old mice)	'treatment*day*time of day' F(11,306)=0.894 p=0.547 with 'treatment*time of day' p=0.047 *	'treatment' p=0.114 'day' p<0.0001* 'time of day' p<0.0001*

	REM sleep (24 months old mice)	'treatment*day*time of day' F(11,306)=0.642 p=0.792 with 'treatment*time of day' p=0.002 *	'treatment' p=0.4 'day' p<0.0001* 'time of day' p<0.0001*
3	Waking (6 months old mice)	'treatment*day*time of day' F (1,72) = 0.0001 p=0.986 with 'treatment*Light-Dark' p<0.0001 *	'treatment' p=0.141 'Light-Dark' p<0.0001 * 'day' p=0.012 *
	NREM sleep (6 months old mice)	'treatment*day*time of day' F (1,72) = 0.034 p=0.855 with 'treatment*Light-Dark' p=0.001 *	'treatment' p=0.013 * 'Light-Dark' p<0.0001 * 'day' p=0.087
	REM sleep (6 months old mice)	'treatment*day*time of day' F (1,72) = 0.353 p=0.554 with 'treatment*Light-Dark' p=0.013 *	'treatment' p=0.002 * 'Light-Dark' p<0.0001 * 'day' p<0.0001 *
3	Waking (18 months old mice)	'treatment*day*time of day' F (1,56) = 0.006 p=0.937 with 'treatment*Light-Dark' p=0.039 *	'treatment' p=0.586 'Light-Dark' p<0.0001 * 'day' p=0.361
	NREM sleep (18 months old mice)	'treatment*day*time of day' F (1,56) = 0.0001 p=0.999 with 'treatment*Light-Dark' p=0.086	'treatment' p=0.415 'Light-Dark' p<0.0001 * 'day' p=0.888
	REM sleep (18 months old mice)	'treatment*day*time of day' F (1,56) = 0.063 p=0.8	'treatment' p=0.482 'Light-Dark' p<0.0001 * 'day' p=0.016 *
3	Waking (24 months old mice)	'treatment*day*time of day' F (1,51) = 0.006 p=0.936	'treatment' p=0.315 'Light-Dark' p<0.0001 * 'day' p=0.003 *
	NREM sleep (24 months old mice)	'treatment*day*time of day' F (1,51) = 0.007 p=0.935	'treatment' p=0.182 'Light-Dark' p<0.0001 * 'day' p=0.002 *
	REM sleep (24 months old mice)	'treatment*day*time of day' F (1,51) = 0.001 p=0.971	'treatment' p=0.653 'Light-Dark' p<0.0001 * 'day' p=0.3
4	NREM sleep	'age*Light-Dark*day' F(2,88)=0.6 p=0.55 with 'age*Light-Dark' p=0.006	'age' p=0.339 'Light-Dark' p<0.0001 * 'day' p=0.024 *
	REM sleep	'age*Light-Dark*day' F(2,88)=0. 593 p=0.555	'age' p=0.634 'Light-Dark' p<0.0001 * 'day' p=0.005 *

5	REM sleep (DLAN only mice)	'age*time of day*day' F (22,480) = 0.366 p=0.997	'age' p<0.0001 * 'time of day' p<0.0001 * 'day' <0.0001 *
6	Cumulative REM sleep (all DLAN mice)	'age*time of day' F(46,480)=0.34 p>0.99	'age' p<0.0001 * 'time of day' p<0.0001 *
7	Waking (6 months old mice)	'treatment*EEG frequency bins' F(29,540)=4.9 p<0.0001 *	'treatment' p<0.0001 * 'EEG frequency bins' p<0.0001 *
	NREM sleep (6 months old mice)	'treatment*EEG frequency bins' F(29,540)=5.93 p<0.0001 *	'treatment' p<0.0001 * 'EEG frequency bins' p<0.0001 *
	REM sleep (6 months old mice)	'treatment*EEG frequency bins' F(29,540)=1.348 p=0.1082	'treatment' p<0.0001 * 'EEG frequency bins' p<0.0001 *
	Waking (24 months old mice)	'treatment*EEG frequency bins' F(29,390)=1.5 p=0.0557	'treatment' p<0.0001 * 'EEG frequency bins' p<0.0001 *
	NREM sleep (24 months old mice)	'treatment*EEG frequency bins' F(29,390)=2.2 p=0.0006 *	'treatment' p<0.0001 * 'EEG frequency bins' p<0.0001 *
	REM sleep (24 months old mice)	'treatment*EEG frequency bins' F(29,390)=1.15 p=0.2786	'treatment' p<0.0001 * 'EEG frequency bins' p<0.0001 *
8	Slow-wave activity (SWA) in NREM sleep (all groups)	'treatment*time of day*day' F (55,1033)=0.093 p>0.99	'treatment' p<0.0001 * 'time of day' p=0.057 'day' p=0.006 *
	Slow-wave activity (SWA) in NREM sleep (6 months old control mice)	'time of day*day' F(8,89)=11.05 p<0.0001 *	'day' p=0.05 'time of day' p=0.93
	Slow-wave activity (SWA) in NREM sleep (18 months old control mice)	F(8,63)=5.48 p<0.0001 *	'time of day' p=0.9995 'day' p<0.0001 *
	Slow-wave activity (SWA) in NREM sleep (24 months old control mice)	'time of day*day' F(8,66)=9.46 p<0.0001 *	'time of day' p=0.9988 'day' p<0.0001 *

8	Slow-wave activity (SWA) in NREM sleep (6 months old DLAN mice)	'time of day*day' F(8,71)=2.447 p=0.0212 *	'time of day' p=0.99 'day' p=0.31
	Slow-wave activity (SWA) in NREM sleep (18 months old DLAN mice)	'time of day*day' F(8,63)=11.59 p<0.0001 *	'time of day' p=0.99 'day' p=0.0026 *
	Slow-wave activity (SWA) in NREM sleep (24 months old DLAN mice)	'time of day*day' F(8,45)=8.388 p<0.0001 *	'time of day' p=0.99 'day' p=0.0576
	Slow-wave activity (SWA) in NREM sleep (6 months old DLAN mice)	'time of day*day' F(8,71)=2.447 p=0.0212 *	'time of day' p=0.99 'day' p=0.31
	Slow-wave activity (SWA) in NREM sleep (18 months old DLAN mice)	'time of day*day' F(8,63)=11.59 p<0.0001 *	'time of day' p=0.99 'day' p=0.0026 *
	Slow-wave activity (SWA) in NREM sleep (24 months old DLAN mice)	'time of day*day' F(8,45)=8.388 p<0.0001 *	'time of day' p=0.99 'day' p=0.0576