Universiteit
Leiden
The Netherlands

Sleep alterations in the course of aging environmental inputs

Panagiotou, M.

Citation
Panagiotou, M. (2020, May 14). *Sleep alterations in the course of aging environmental inputs*. Retrieved from https://hdl.handle.net/1887/87898

Version:	Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/87898

Note: To cite this publication please use the final published version (if applicable).

Cover Page

Universiteit Leiden

Leiden University Repository

The handle http://hdl.handle.net/1887/87898 holds various files of this Leiden University dissertation.

Author: Panagiotou, M.
Title: Sleep alterations in the course of aging environmental inputs
Issue Date: 2020-05-14

Chapter 7

High-caloric diet and sleep in aged mice

Chronic high-caloric diet accentuates age-induced sleep alterations in mice

Maria Panagiotou, Tom Deboer

Laboratory for Neurophysiology, Department of Cell and Chemical Biology, Leiden University Medical Center, The Netherlands

Published in Behavioral Brain Research 2019 Apr 19;362:131-139

Abstract

Obesity and sleep disturbances comprise major health problems which are likely interrelated. Diet-induced obesity in young mice has been demonstrated to lead towards an altered sleep homeostasis. In the current study, we investigated the effect of chronic (12 weeks) high-caloric diet (HCD, 45% fat) consumption on sleep and the sleep electroencephalogram (EEG) in young and older mice (6-month-old, n=9; 18-month-old, n=8 and 24-month-old, n=4) and compared with age-matched controls on normal chow (n=11, n=9 and n=9 respectively). Half of the 24-month-old mice did not cope well with HCD, therefore this group has a lower n and limited statistical power. We recorded EEG and the electromyogram for continuous 48-h and performed a 6-h sleep deprivation during the second day. In aged HCD fed mice (18 months old) compared to young, an aging effect was still evident, characterized by decreased waking and increased NREM sleep in the dark period, decreased REM sleep during the light period, as well as increased slow-wave-activity (SWA, EEG power in NREM sleep in 0.5-4.0 Hz). Additionally, aged HCD treated mice showed increased NREM sleep and decreased waking, compared to age-matched controls, denoting an enhanced aging phenotype in the sleep architecture. Notably, an overall increase was found in the slow component of SWA (0.5-2.5 Hz) in aged HCD fed mice compared to age-matched controls. Our data suggest that the effect of aging is the dominant variable irrespective of diet. However, a synergistic effect of aging and diet is noted indicating that chronic HCD consumption exacerbates age-associated sleep alterations.

Keywords: obesity, aging, sleep disturbances, slow-wave activity

Introduction

Obesity is nowadays a common characteristic seen across all ages. It raises the risk of several diseases that could lead to premature mortality. For example, the risk of dying from heart disease is increased irrespective of aging, and obesity is thought to underlie age-associated insulin resistance [1, 2].
Obesity and sleep are likely interrelated and may share common pathways [3, 4, 5, 6, 7]. Sleep in people with obesity might appear disturbed and problems falling asleep or waking up often exist. An age-specific prevalence for daytime sleepiness, a frequently reported feature of people with obesity, has been observed [8]. In particular, a linear decline of daytime sleepiness was found in ages from 30 to 75 years old, whereas a higher prevalence of sleepiness was found in people with obesity in ages outside of this limit (<30 and >75 y. o.). In addition to human studies, laboratory animals have been used to study sleep in obesity. Young mice chronically fed with high-caloric diet (HCD), in order to develop obesity, showed sleep architecture alterations [6, 9, 10, 11, 12], slower build-up of sleep pressure and an altered sleep homeostasis [12]. Although the HCD effect on sleep characteristics has received some attention in childhood, adolescent and young adult life, it has not been extensively explored in the course of aging.
Sleep is regulated by two main processes, a circadian process governed by the internal biological clock, and a sleep homeostatic process which is dependent on prior waking and sleep [13, 14]. In mammals, the homeostatic sleep process is thought to be reflected in the NREM sleep electroencephalographic (EEG) slow-wave activity (SWA, EEG power density between 0.75-4.0 Hz) [13, 14].
Aging leads to a redistribution of body fat, with a stabilization or enhancement of visceral fat and fat deposition into tissues such as liver, muscle, and bone [15], similar to the changes seen during HCD consumption [16]. In healthy aged mice, sleep patterns are characterized by increased non-rapid-eye-movement (NREM) sleep as well as increased SWA levels [17, 18, 19, 20, 21, 22, 23]. The effect of HCD as the exclusive diet in aged mice on sleep and the sleep EEG has not been examined, and could give important insights in the effect of diet on sleep in later life.
Therefore, in this study, we investigated the effect of chronic (12 weeks) HCD consumption on sleep architecture and the sleep EEG in young (6 months old) and aged (18 months and 24 months old) C57BL/6J mice during 24-h baseline (BL) recordings, as well as after 6-h of sleep deprivation (SD). We found that the effect of aging on sleep parameters is pronounced irrespective of diet, rendering aging the dominant variable over HCD regarding sleep alterations. Nevertheless, diet and aging likely act synergistically leading to the notion that chronically consumed HCD accentuates age-induced sleep changes.

Materials and methods

Animals

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.
Male C57BL/6JOlaHsd mice of three age groups (6-, 18- and 24-month-old, n=48) (Har-

lan, Horst, the Netherlands) were used for this study. All mice were fed with normal chow (11% fat, 27% protein, 61% carbohydrate, Special Diet Services, UK) before entering the study. Three months before the surgeries (at the age of 3, 15, 21 months for the 6-, 18- and 24-month-old group respectively), mice were partitioned into two groups, the control, in which mice were kept on normal chow diet, and the HCD fed group, in which mice were fed exclusively with high-caloric food, in a pellet form, being very similar to control chow (45% fat mainly derived from lard, 35% carbohydrate, 20% protein; D12451, Research Diet Services, The Netherlands) for 12 weeks. The mice were individually housed under controlled conditions (12:12 h light:dark cycle; lights on at 10:00) with food and water ad libitum in a temperature controlled room (21-22°C).
Originally, the 24-month-old HCD treated group was larger (n=9), however, approximately half of the mice in this age group did not cope well with the HCD that was introduced in their life when they were 21 months old and had severe side effects that led to their premature sacrifice (5 out of 9 mice). Since this did not occur at the age-matched control mice (or any other group used for this study), we attribute the health problems of the aged mice to the altered diet. The remaining 24 months old mice (n=4) did not gain the same amount of weight as the 18 and 6 months old mice that received the same treatment, albeit the amount of food consumed did not seem to differ. Nevertheless, although not delving deeply into the data for this group, we are reporting mainly in the supplementary material and briefly commenting on the obtained results throughout the current study, for research purposes.

Surgeries

On the day of the surgery, 6-month-old HCD fed animals (n=9) weighed on average 47.6 g ($\pm$ 0.8), whereas, their age-matched control animals (n=11) weighed 30.7 g ($\pm$ 0.8), 18-month-old HCD fed animals (n=7) weighed on average 45.43 g ($\pm$ 1.9), whereas their age-matched controls (n=8) weighed 33.5 g ($\pm$ 0.7), and 24-month-old HCD fed animals (n=4) weighed on average 35 g ($\pm$ 2.1), whereas their age-matched controls (n=9) weighed 32.7 g ($\pm$ 1). 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 [12, 18, 24]. 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.

Vigilance state and 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 [12, 18, 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 food and water availability. Before starting the experiment, the animals were allowed to adjust to the experimental conditions for a week. Subsequently, a BL day was recorded, starting at lights on. At the start of the second day, six hours of sleep-deprivation (SD) were conducted. Similar to earlier studies [12, 18, 24, 25], during SD in the experimental chambers, animals were kept awake by gentle handling in which introduction of new food pellets or nesting material and light noises were involved. EEG and EMG were recorded continuously for 48-h, during BL (24-h), SD (6-h), as well as for 18-h 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 [12, 18, 24, 25, 26]. 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. Number of episodes was computed in each vigilance state that were partitioned into ten exponentially increased duration bins from 4s to >1024s similar to [26, 27, 28]. 12-h light and dark (L1, D1, L2, D2 for light and dark periods of the first and second day respectively) mean values of vigilance states were analyzed by two-tailed unpaired t-tests for each period to determine the effect of treatment. Note that, to test the effect of the experimental day, L2 corresponds to the 6-h after SD and is compared to the corresponding 6-h of the BL. Three-way ANOVA was performed to investigate potential alterations on vigilance states, vigilance state episode frequency in duration bins, and 2-h time bins of EEG power density in NREM sleep with main factors 'treatment', 'age', 'day' 'Light-Dark', 'time of day', 'duration bin', 'EEG frequency bin' and '2-h intervals'. Two-way ANOVA was performed regarding the EEG power density of the vigilance states with main factors 'age' and 'EEG frequency bin'. Regarding the Slow/Fast/Overall SWA levels one-way ANOVA was performed with main factor 'treatment'. When appropriate, paired and unpaired post-hoc Bonferroni-corrected student's t-tests were applied to determine the effects of treatment, age and SD. Although novel analysis is included, the 6-month-old group including both high-caloric-diet fed and control mice is the data set published earlier in Panagiotou et al., 2018 [12]. However new analysis on this data set is added here according to the aforementioned tests, that includes the comparisons to the aged groups, the more detailed analysis of the absolute spectra and slow-wave activity as well as the number of vigilance state episodes.

Results

Sleep architecture

HCD and sleep in young and aged mice

Sleep architecture was differentially altered by chronic HCD in young mice (Fig.1 and

Fig.2 left panels) that was elaborately investigated and described previously (data for young mice already published in [12]). HCD mainly induced an increase in REM sleep during the light period without affecting the amount of waking or NREM sleep. By computing the number of episodes, this REM sleep difference was found to be mainly caused by an increase in the short and middle duration REM sleep episodes (8-32s) (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (*Supplementary material*, Fig. S1).

18 months old HCD fed mice showed increased NREM sleep during the 24-h BL and decreased waking and no difference in REM sleep compared to their age-matched control mice (Fig. 1, right panel) (18 months old mice: Waking, p=0.028, NREM sleep, p=0.027, REM sleep, p=0.307). The difference in the 18 months old HCD fed mice arises from a decrease in waking and increase in NREM sleep in both 12-h BL light and dark periods (Fig. 1) (Waking, unpaired t-test for L1: p=0.02, D1: p=0.044, L2: p= 0.003 D2: p= 0.013, NREM sleep, unpaired t-test for L1: p=0.0199, D1: p=0.042), L2: p= 0.001, D2: p= 0.012). In contrast, no significant 24- and 12-h effects were found in the 24 months old mice compared to their age-matched controls (*Supplementary material*, Fig. S2A).

The effects of SD were evident in the 18 months old control mice and 24 months control and HCD fed mice (post-hoc two-tailed paired t-tests between BL and after SD time, *Supplementary Material* Table S1) (Fig. 1 and *Supplementary material*, Fig.S2A). Particularly, following SD in the 18 months old control mice, an increase in NREM sleep and decrease in waking was found during the 6-h of the light period, compared to the corresponding 6-h of the BL light period. Additionally, an increase in NREM sleep and decrease in waking was found in both periods following SD in the 24 months old HCD fed mice and 24 months old controls.

The effect of chronic HCD consumption on sleep architecture is also evident in the time course of 2-h values over the day (Fig. 2), where more NREM sleep and less waking is shown in the 18 months old HCD fed mice, effects not evident in the 24 months old age group (*Supplementary material*, Fig.S2B) (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1). As a function of age, effects of SD were more subtle on vigilance states, with 24 months old mice (both control and HCD fed) no longer showing any alterations (p>0.05).

In addition, by computing the number of episodes, it was found that 18 months HCD fed mice had more very short waking episodes (4s) in both BL light and dark periods, and more very short NREM sleep episodes (4s) and less middle (16s) REM sleep episodes in the BL light period as compared to age-matched controls (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (*Supplementary material*, Fig. S3). 24 months old HCD fed mice showed more subtle differences compared to age-matched controls having less short (8s) waking episodes and less middle-to-long (128s) NREM sleep episodes in BL light period (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (*Supplementary material*, Fig. S4).

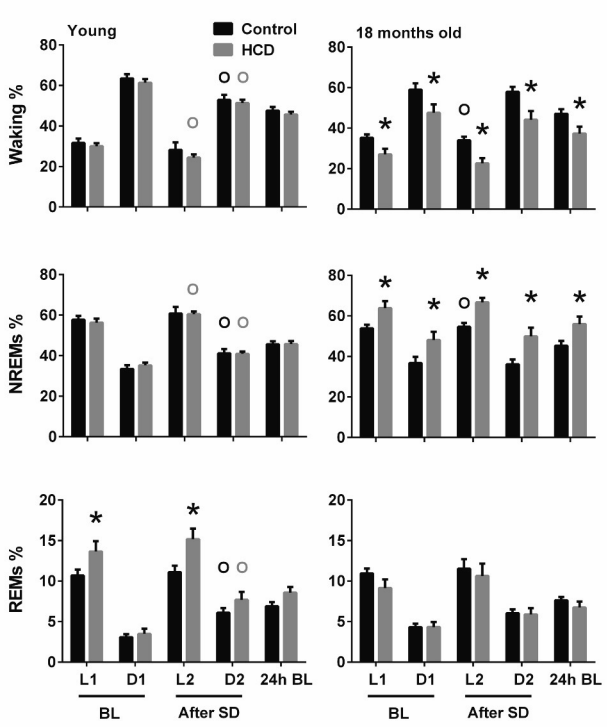

Figure 7.1: Light-dark distribution of each vigilance state (Waking, NREM and REM sleep) during the baseline day (BL: L1, D1) and after sleep deprivation (after SD: L2, D2). Bar plots represent mean ($\pm$ SEM) values (L1, D1, D2 correspond to 12-h values and L2 to 6-h values for the recovery period after SD, for light and dark 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 (n=11, n=9 for young and 18 months old mice, black bars) and high-caloric diet fed mice (HCD, n=9 and n=7 for young and 18 months old mice, gray bars). Note that for comparisons between BL and recovery day 6-h of L2 were compared with the corresponding 6-h of the baseline light period. Asterisks indicate significant differences between corresponding age groups (unpaired t-tests for each period, $p<0.05$) and black and gray circles indicate significant differences between recovery and BL day for control and HCD fed mice respectively (post-hoc paired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment', 'Light-Dark', 'day').).

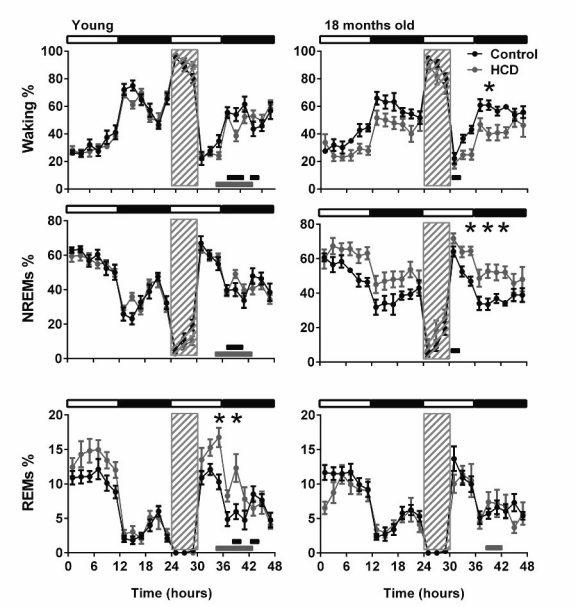

Figure 7.2: Time course of vigilance states (Waking, NREM sleep and REM sleep), for 24-h baseline (BL), 6-h sleep deprivation (SD, hatched bar) and 18-h recovery for young and 18 months old control and high-caloric diet (HCD) treated mice (left and right panels respectively). Curves connect 2-h mean (± SEM) values. The black and white bars above each graph indicate the light-dark cycle. Black asterisks at the top of each graph represent significant differences between the groups across the 48-h period and black and gray bars at the bottom of each graph significant differences between recovery and BL day for each 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').

Aging and sleep in HCD fed mice

In healthy aged mice, more NREM sleep, stemming from more longer-duration episodes, and less waking during the dark period as well as less REM sleep at the end of the light period has been shown earlier compared to young mice [17, 18, 19, 20, 21, 22, 23]. In the current study, we detected alterations in the sleep architecture of HCD fed mice of the three different age groups, which were mainly found between the young and the aged groups. These consisted of decreased waking during BL dark period, increased NREM sleep in both 12-h BL light and dark periods as well as after SD, and decreased REM sleep in the both light periods, whereas similar data were obtained in the two aged groups for all vigilance states with no significant differences (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (Fig. 3A and *Supplementary material*, Fig.S5A).
Regarding the number of episodes, we found significant differences between young and both aged groups of mice that were chronically fed with HCD (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (Fig. 3B and *Supplementary material*,

Fig.S5B). During the BL light period, 18 months old HCD fed mice had more brief awakenings (1-4s) and both aged groups had less of the longest category waking episodes (>1024s) compared to young HCD fed mice. Additionally, during the 12-h light period less middle-to-long (64s) and more long (256s) NREM sleep episodes, as well as less middle-to-long (128s) REM sleep episodes were found in the 18 months old HCD fed mice compared to the young animals. During the BL dark period, aged HCD fed mice had more short, middle and long waking and NREM sleep episodes, albeit less very long waking episodes compared to the young HCD fed mice. The number of episodes in waking, NREM and REM sleep did not differ in both 18 months and 24 months old HCD treated when compared to age-matched control mice.

EEG power density (0.5 - 25 Hz)

To study the effect of chronic HCD consumption on the EEG in the course of aging, we calculated the spectral distribution of absolute EEG power density in the frequencies between 0.5 and 25 Hz (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (Fig. 4 and Supplementary material, Fig.S5C). Both groups of aged HCD fed mice had higher EEG power density in the slow frequencies (between 0.5-4 Hz in the 18 and 2-7 Hz in the 24 months old mice) during waking, as compared to young HCD fed mice. The NREM sleep EEG spectra in aged HCD fed animals were characterized by a pronounced increase in power density in the slow frequencies (0.5-3 Hz in the 18 and 1-7 Hz in the 24 months old mice) compared to the young HCD fed mice. REM sleep EEG spectra showed higher EEG power density in the 18 months old HCD fed mice in the faster theta frequencies (between 7-8 Hz) and in the 24 months old higher around 2.5 Hz and in the theta frequencies (between 6-9 Hz) compared to young HCD fed mice. Additionally, higher EEG power density in NREM sleep around 3 and 6-7 Hz as well as in REM sleep around 6-8 Hz was noted in the 24 months old compared to 18 months old HCD fed mice. In contrast, EEG power density in young HCD mice tended to be lower in the frequencies below 5 Hz, compared to control. Similar EEG power density spectra were obtained in 18 months and 24 months old HCD fed mice as compared to age-matched controls during waking, NREM and REM sleep. Both aged HCD fed groups showed no significant differences across the vigilance states in the power spectra compared to age-matched controls, albeit a subtle decrease in the REM sleep spectrum in the 18 months old HCD fed mice compared to control around 6 Hz and 22 Hz (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (*Supplementary material*, Fig. S6).
As a follow up, we computed the EEG power density in NREM sleep in the frequencies between 0.5 and 25 Hz in 2-h time bins across BL light and dark periods and detected an altered time course in EEG activity between groups, particularly in the slow frequencies (0.5-5 Hz) during the light period (post-hoc two-tailed unpaired t-tests, *Supplementary Material* Table S1) (*Supplementary material*, Fig. S7).

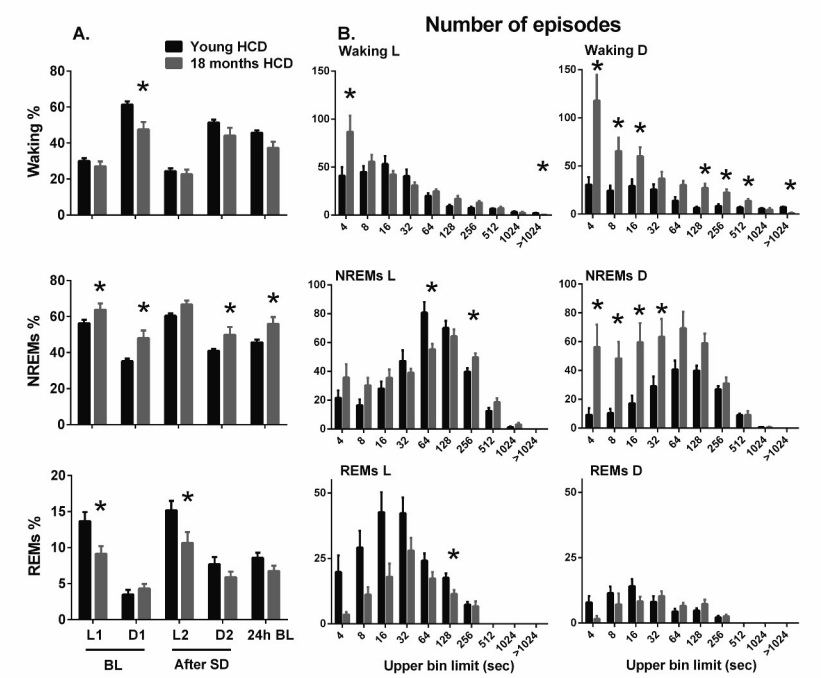

Figure 7.3: Light-dark distribution of each vigilance state (Waking, NREM and REM sleep) during the baseline day (BL: L1, D1) and after sleep deprivation (after SD: L2, D2) and histogram of vigilance state episodes frequency. A. Bar plots represent mean (± SEM) values (L1, D1, D2 correspond to 12-h values and L2 to 6-h values for the recovery period after SD, for light and dark periods during the 48-h recordings respectively) and 24-h values of baseline recordings (24-h BL) for Waking, NREM and REM sleep for young and 18 months old high-caloric-diet (HCD) fed mice (n=9 and n=7, black and gray bars respectively). Note that for comparisons between BL and recovery day 6-h of L2 were compared with the corresponding 6-h of the baseline light period. B. Episodes were partitioned in ten exponentially increased duration bins (upper bin limit from 4 to >1024s) in baseline light and dark periods. Asterisks indicate significant differences from the young HCD fed mice (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'age', 'Light-Dark', 'day').

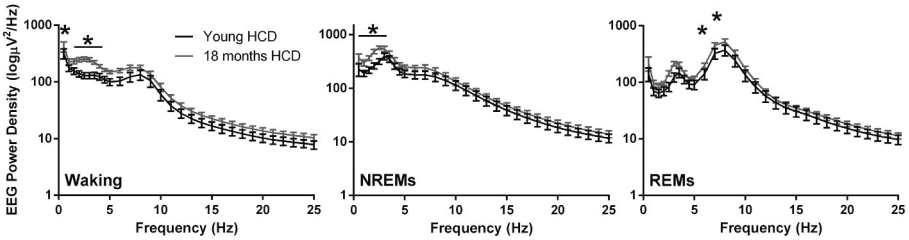

Figure 7.4: Electroencephalographic (EEG) power density in Waking, NREM and REM sleep for young and 18 months old high-caloric-diet (HCD) fed mice (log $\mu V^2/Hz$, 0.5-25 Hz). Black asterisks indicate significant differences between 18 months old and young HCD fed mice (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, p<0.05 after significant ANOVA, main effects 'age', 'EEG frequency bin').

Slow-wave activity in NREM sleep (SWA)

The spectral data suggested that HCD specifically affects EEG SWA during NREM sleep. To investigate this further, we computed the absolute NREM SWA levels in all groups in 2-h intervals across the 48-h of recordings (Fig. 5 and *Supplementary material*, Fig.S2C) (*Supplementary Material* Table S1). We found that the effect of HCD differs across aging, where 18 months old HCD fed mice showed no alterations, 24 months old HCD fed mice showed an increase and young HCD mice an overall decrease in SWA levels compared to their age-matched controls (One-way ANOVA, factor 'treatment', p<0.0001; post-hoc Bonferroni t-tests: p<0.05). Interestingly, the three HCD fed groups significantly differed from each other (post-hoc Bonferroni tests: p<0.0001) showing an increase in SWA as a function of age.

Additionally, taking into account the results from figure S7, where significant differences in the slow frequency range, as well as an altered behavior between slow (0.5-2.5 Hz) and fast (2.5-4Hz) slow-waves [29] were detected, we computed the time course of slow and fast SWA for all groups (Fig. 5). An overall significant treatment effect was noted in the slow and fast SWA range (One-way ANOVA, factor 'treatment', Slow SWA: p<0.0001, Fast SWA: p<0.0001). Post-hoc multiple comparisons across treatment groups revealed that the 18 months old HCD fed mice had increased slow SWA levels, whereas 24 months old HCD fed mice showed an increase in both the slow and fast SWA, and therefore an overall increase, compared to age-matched controls. In contrast, the decrease in overall SWA seen in young HCD mice emerged from a decrease of the slow SWA, whereas no change was found in the fast SWA. Therefore, slow and fast slow-waves are differentially affected by age and diet.

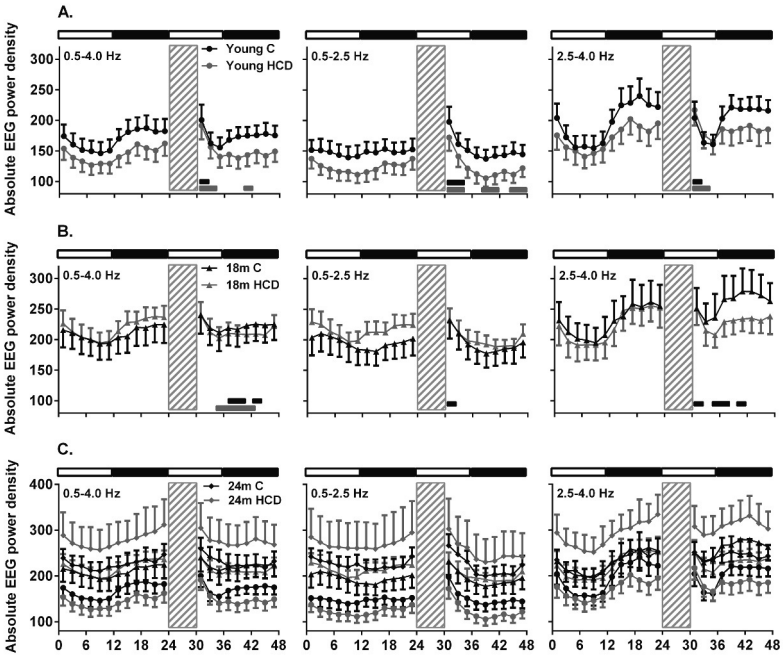

Figure 7.5: Time course of absolute electroencephalographic (EEG) power density ($\mu V^2/Hz/0.5Hz$) for the overall slow-wave activity range (SWA, 0.5-4 Hz) in non-rapid-eye movement (NREM) sleep (left graph), for the slow SWA 0.5-2.5 Hz (middle graph) and the fast SWA 2.5-4 Hz (right graph) for 24-h baseline (BL), 6-h sleep deprivation (SD, hatched bar) and 18-h recovery for young (A), 18 months old (B) and 24 months old (C) control and high-caloric diet fed (HCD) mice. Bars at the bottom of each graph indicate significant differences between recovery and BL day for each age group (post-hoc paired t-tests with Bonferroni multiple comparisons correction p<0.05, after significant ANOVAs, main effects 'day' and 'time of day'). Overall decreased EEG SWA levels were revealed in the young HCD fed mice (0.5-4 Hz and 0.5-2.5 Hz) and overall increased levels in 18 months (0.5-2.5 Hz) and 24 months old HCD fed mice (0.5-4 Hz, 0.5-2.5 Hz and 2.5-4Hz) compared to control mice (one-way ANOVA, factor 'treatment'; p<0.05).

Discussion

In this study, the effect of chronic HCD consumption was investigated in the course of aging. Both aged HCD fed groups demonstrated similar sleep architectural patterns that significantly differed from the young HCD fed mice, consisting of increased NREM sleep, decreased waking and decreased REM sleep, as well as absence of very long waking episodes. Additionally, higher EEG power density during BL recordings was obtained in the slow frequencies of all vigilance states in both aged HCD fed mice compared to young HCD fed mice. Compared to age-matched controls, 18 months old HCD fed mice showed increased NREM sleep and decreased waking in both light and dark periods, whereas 24 months old HCD fed mice showed no alterations. Notably, the slow and

fast SWA component was differently affected in the course of aging in HCD fed mice. Decreased levels of the slow SWA were found in the young HCD fed mice, whereas increased levels were found in the 18 months old HCD fed mice, while for the 24 months old a general increase in both slow and fast SWA was found. Our data show that aging is the dominant variable inducing alterations in the sleep architecture and the sleep EEG. However, the combined effect of aging plus diet seems to provoke detrimental effects leading to exacerbated aging patterns in sleep and the sleep EEG.

Sleep architecture

Recent research showed that chronic HCD consumption in young mice leads to altered sleep homeostasis with a slower build-up rate of sleep pressure, increased REM sleep during the light period, an increase in consecutive NREM-REM sleep cycles and changes in waking, NREM, and REM sleep episode number and duration throughout the 24-h [6, 9, 10, 11, 12]. During natural aging, mice demonstrate increased NREM sleep during the dark period emerging from an increase in long NREM sleep and short waking episodes, and decreased REM sleep during the light period [17, 18, 19, 20, 21, 22, 23]. In accordance, aged mice show augmented SWA levels [17, 18]. Similar effects were noted in both aged HCD groups in the current study compared to the young HCD fed group, where aging characteristics were evident in the sleep architecture, including increased NREM sleep, decreased waking and decreased REM sleep, as it is shown earlier [17, 18, 19, 20, 21, 22, 23]. However, compared to age-matched controls, 18 months old HCD fed mice demonstrated an accentuated aging phenotype in sleep architecture, characterized by a further increase in NREM sleep and decrease in waking.
Total fat mass is known to peak at middle age in both humans and rodents [15]. In addition, older people have elevated levels of abdominal adipose tissue compared to young individuals [15, 30, 31], which has been characterized as a risk factor for several diseases. Adipose tissue from agouti mice, which are genetically obese, showed features of premature aging, higher levels of reactive-oxygen-species (ROS) and DNA damage [32]. In our study, the notion is developed that obesity and aging may act synergistically, affecting sleep architecture. This may occur through the increasing total fat mass levels as well as adipose tissue likely induced by both obesity and aging in the 18 months old mice, which coincide to 50-60 y.o. in humans, intensifying this way the already age-induced sleep architectural characteristics.
Insufficient orexin signaling has been implicated in several medical conditions including obesity [3], and neurodegenerative diseases [33, 34]. Being characterized as a regulator of vigilance states, energy homeostasis, as well as the reward system and feeding [35, 36], orexin may be implicated in the regulation of sleep in young HCD fed mice, as increased levels of REM sleep and an increased number of NREM-REM sleep cycles were found [12], similar to what occurs in narcolepsy [37]. In the course of aging, a decline in the orexin system has been demonstrated in many species, including mice [38, 39], rendering orexin an important factor underlying age-related metabolic and sleep/wake cycle alterations. We suggest that chronic HCD may influence the orexin system, however, this effect is expressed differently in the sleep of young and aged mice, since aging can act as a synergistic factor to obesity.
Furthermore, adenosine kinase has been found to be increased in the cerebral cortex of

old animals [40]. Under a high-fat diet, it has also been shown that levels of adenosine monophosphate-activated protein kinase (mAMPK) decline [41]. In addition, blocking adenosine kinase is likely to prolong sleep and further increase SWA in the EEG [42]. Although the specific mechanisms are not known, it seems that the adenosinergic system could play a role in the way HCD affects sleep architecture and general sleep function in the course of aging.

EEG power density and slow-wave activity

When compared to the young HCD fed mice, aged HCD fed mice demonstrate alterations in the waking, NREM and REM sleep spectra, in accordance with the aging sleep and EEG phenotype. In particular an increase in EEG SWA in NREM sleep was observed in the course of aging. These changes are consistent with a previous study, suggesting that sleep pressure is increased in aged compared to young mice [18]. The question remains whether HCD in aging adds further effects.
We therefore computed the changes in the EEG power density spectra between 0.25-25.0 Hz in 2-h time bins (*Supplementary Material*, Fig. S7), and found the most pronounced differences between the groups between 0.5-5 Hz. In addition, during NREM sleep we observed differences in the time course of the slow (0.5-2.5 Hz) and fast (2.5-4.0 Hz) frequency bands within the slow-wave range. It is known that SWA can be partitioned in two different bands according to a different origin of the waves, first the slow component, with frequencies around 0.5-2.5 Hz which correspond to cortico-cortical connections, and second the faster frequencies within 2.5-4 Hz which correspond to thalamo-cortical connections [29]. Therefore, we investigated the time course of the overall SWA (0.5-4 Hz), as well as of the slow and fast frequency ranges for each group (Fig. 5). The data show increased SWA levels in aged mice irrespective of diet, confirming earlier findings from our group [18]. This shows that aging is the prevailing parameter influencing SWA. Interestingly, it seems that HCD acts synergistically with aging differently affecting the slow and fast component of SWA. In the case of the 18 months old mice, the slow component of SWA showed an overall increase under influence of HCD, whereas the fast component remained unaltered, leading to no significant change in the overall SWA levels. In contrast, 24 months old HCD fed mice showed an increase in both the slow and fast component which resulted in an overall increase in SWA. The increase in the slow component of SWA in 18 months old HCD fed mice and the increase in both the slow and fast components in the 24 months old HCD fed mice indicating an amplification of the aging phenotype by HCD. In addition, it is important to note that young and aged mice respond differently to chronically consumed HCD as reflected in the SWA levels. We show that the slow component increased in both aged groups fed HCD, whereas young HCD fed mice had lower SWA levels, particularly in the slow SWA range.
Brain aging is still unavoidable, however, a line can be drawn between successful brain aging which is important for increased longevity and health, and pathological brain aging, which can accelerate health deterioration. Recently, it was shown that, during healthy aging, in mice slow-wave characteristics alter, probably reflecting brain connectivity changes [18]. It has also been proposed that during normal brain aging imbalances of neurotransmitters and increases in inflammatory signaling and oxidative stress may occur [15]. High-fat diet has been demonstrated to promote insulin resistance and

metabolic dysfunction in both humans and rodents, however the impact on brain function remains poorly understood [6, 43, 44, 45, 46]. It has been proposed that HCD could impede neuronal plasticity and modulate astrocytic and microglial signaling [47, 48, 49]. Additionally, it has been hypothesized that the slow SWA component, emerging from the cortico-cortical connections in the brain, involves glial activities [50, 51]. Theories have been developed linking astrocytes, metabolism and sleep via mechanisms such as the neuronal-glial 'lactate shuttle', according to which synaptic glutamate release triggers lactate production and release in astrocytes, evident in brain lactate levels, that increase during wakefulness and decrease during NREM sleep in parallel with NREM SWA [48, 52, 53, 54, 55]. In the current study, we show that particularly the slow component of SWA was altered under influence of HCD in aged mice, intensifying the aging effects. Therefore, the data suggest that HCD may impact cortical brain connectivity through a glial pathway. In contrast, the thalamocortical pathway underlying the fast SWA component is clearly influenced by aging, however, it is differently affected by HCD across the age groups. The thalamus and its associated network may play a different role in the interaction between HCD and aging. Compared to young controls, hypocretin receptor 2 levels were found to be declined in the thalamus of 24 months old mice but not in 18 months old [38]. We therefore suggest that alterations in fast SWA are the result of an interacting influence of HCD and aging on thalamo-cortical activity.

The changes caused by HCD in mice resemble metabolic disturbances observed in humans, however, HCD consumption can also promote novel metabolic perturbations, as well as accelerate individual aspects of age-related metabolic disturbances [15, 56]. In our results, it seems that altered brain connectivity, apparent in the EEG SWA, is likely synergistically impacted by age and diet.

Furthermore, chronic HCD consumption may play an important role in regulating age-related increases in oxidative stress. It was demonstrated that lipoprotein metabolism and oxidant/antioxidant status were altered in obesity, irrespective of age. Nevertheless, obesity-related lipid and lipoprotein alterations were attenuated, whereas, oxidative stress was worsened in older adults [57]. In aged HCD-induced obese mice, HCD exacerbated age-related oxidative stress in the brain via increased NADPH oxidase (NOX), rendering NOX activity a player in the ability of HCD to detrimentally affect brain homeostasis in aged individuals, likely associated with feed-forward cascades of oxidative stress and brain inflammation [58]. In the current study, we find that chronic HCD consumption amplifies aging effects on the slow component of NREM sleep SWA levels of aged mice. Recent studies implicate aberrant or excessive activation of microglia in the pathogenesis of brain aging, which could also account for the augmented levels found when aging is combined with HCD. Further research is undoubtedly needed regarding the relation of glial activation and associated pathways with chronic obesity and brain wave alterations in order to verify our aforementioned hypotheses.

A limitation of the present study is the unsuccessful chronic HCD treatment of the 24 month old group. Approximately 50% of the mice long-term fed with HCD were euthanized because of serious health problems (5 out of 9). C57BL/6 mice are known to express a multifactorial age-related susceptibility to plasma lipid levels, after dietary intake of high fat, rendering this strain susceptible to lipid-related vascular complications such as atherosclerosis [59, 60]. Additionally, an age-dependent change in hepatic lipid and antioxidant metabolism inducing increased susceptibility to lipid peroxidation has

been noted [61, 62]. Therefore, chronic HCD, starting above 18 months of age, is likely to lead to severe health problems as seen in the present study. Therefore, the results and conclusions from the 24 months old group should be treated with caution as the number of animals included is too low for a proper statistical analysis.

Concluding Remarks

It has already been established that the deterioration of structure and function of organs in aging is associated with oxidative stress, genetic instability and disruption of homeostatic pathways [63]. Obesity has been associated with a wide range of chronic diseases that could potentially lead to premature mortality. There are similarities in metabolic dysregulation in aging and obesity, and, thus, it is likely that these conditions share similar pathways. In addition, the brain comprises one of the most crucial sites at the intersection of age and metabolic dysfunction, since cognitive dysfunction and increased risk of dementia constitute debilitating deficits. In our study, we show that, HCD influences sleep differently in the course of aging. Although aging is the prevailing parameter, sleep architecture and the sleep EEG are also affected when HCD is introduced, indicating that the combination of chronic HCD and increased age impacts sleep and brain health. Notably, the slow SWA levels were altered in all age groups under HCD indicating general brain connectivity changes due to this diet, albeit in opposite directions in young and aged mice. Taking the opposite direction effect into account, as well as the attenuated effect of prolonged waking on subsequent EEG SWA in NREM sleep in the young HCD fed mice compared to controls [12], we can conclude that: short-term benefits emerge by consuming HCD at a young age, lowering the burden of the sleep debt incurred, however with increasing age this effect is diminished and faster brain aging is induced instead.

Acknowledgements

This work was supported by a grant from the Dutch Technology Foundation (STW to T. Deboer).

Competing financial interests

The authors declare no conflict of interest.

Data Accessibility Statement

The authors deposit supporting information and datafiles to Figshare for data archiving https://figshare.com/s/b1a96e3601b05c86d423

Bibliography

[1] Amati F, Dube JJ, Coen PM, Stefanovic-Racic M, Toledo FG & Goodpaster BH (2009) Physical inactivity and obesity underlie the insulin resistance of aging. Diabetes Care, 32, 1547-1549.

[2] Thorpe RJ Jr & Ferraro KF. (2004) Aging, Obesity, and Mortality: Misplaced Concern About Obese Older People? Res. Aging, 26, 108-129.

[3] Knutson, K.L. & Van Cauter, E. (2008) Associations between sleep loss and increased risk of obesity and diabetes. Ann. NYAcad. Sci., 1129, 287-304.

[4] Laposky, A.D., Bass, J., Kohsaka, A. & Turek, F.W. (2008) Sleep and circadian rhythms: key components in the regulation of energy metabolism. FEBS Lett., 582, 142-151.

[5] Vgontzas, A.N., Bixler, E.O., Chrousos, G.P. & Pejovic, S. (2008) Obesity and sleep disturbances: meaningful sub-typing of obesity. Arch. Physiol. Biochem., 114, 224-236.

[6] Mavanji, V., Billington, C.J., Kotz, C.M. & Teske, J.A. (2012) Sleep and obesity: a focus on animal models. Neurosci. Biobehav. Rev., 36, 1015-1029.

[7] St-Onge, MP. (2013) The role of sleep duration in the regulation of energy balance: effects on energy intakes and expenditure. J. Clin. Sleep Med., 9, 73-80.

[8] Bixler, E.O., Vgontzas, A.N., Lin, H.M., Calhoun, S.L., Vela-Bueno, A. & Kales, A. (2005) Excessive daytime sleepiness in a general population sample: the role of sleep apnea, age, obesity, diabetes, and depression. J. Clin. Endocrinol. Metab., 90, 4510-4515.

[9] Jenkins, J.B., Omori, T., Guan, Z., Vgontzas, A.N., Bixler, E.O. & Fang, J. (2006) Sleep is increased in mice with obesity induced by high-fat food. Physiol. Behav., 87, 255-262.

[10] Guan, Z., Vgontzas, A.N., Bixler, E.O. & Fang, J. (2008) Sleep is increased by weight gain and decreased by weight loss in mice. Sleep, 31, 627-633.

[11] Luppi, M., Cerri, M., Martelli, D., Tupone, D., Del Vecchio, F., Di Cristoforo, A., Perez, E., Zamboni, G. & Amici, R. (2014) Waking and sleeping in the rat made obese through a high-fat hypercaloric diet. Behav. Brain Res., 258, 145-152.

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

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

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

[15] Uranga RM, Bruce-Keller AJ, Morrison CD, Fernandez-Kim SO, Ebenezer PJ, Zhang L, Dasuri K & Keller JN. (2010) Intersection between metabolic dysfunction, high fat diet consumption, and brain aging. J. Neurochem., 114, 344-361.

[16] Park S, Kim YW, Kim JY, Jang EC, Doh KO & Lee SK. (2001) Effect of high fat diet on insulin resistance: dietary fat versus visceral fat mass. J. Korean Med. Sci., 16, 386-390.

[17] McKillop LE, Fisher SP, Cui N, Peirson SN, Foster RG, Wafford KA & Vyazovskiy VV. (2018) Effects of Aging on Cortical Neural Dynamics and Local Sleep Homeostasis in Mice. J. Neurosci., 38, 3911-3928.

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

[19] Farajnia S, Michel S, Deboer T, vanderLeest HT, Houben T, Rohling JH, Ramkisoensing A, Yasenkov R & Meijer JH. (2012) Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. J. Neurosci., 32, 5891-5899.

[20] Hasan S, Dauvilliers Y, Mongrain V, Franken P & Tafti M. (2012) Age-related changes in sleep in inbred mice are genotype dependent. Neurobiol. Aging, 33, 195-e13.

[21] Banks G, Heise I, Starbuck B, Osborne T, Wisby L, Potter P, Jackson IJ, Foster RG, Peirson SN & Nolan PM. (2015) Genetic background influences age-related decline in visual and nonvisual retinal responses, circadian rhythms, and sleep. Neurobiol. Aging, 36, 380-393.

[22] Welsh DK, Richardson GS & Dement WC. (1986) Effect of age on the circadian pattern of sleep and wakefulness in the mouse. J. Gerontol., 41, 579-586.

[23] Colas D, Cespuglio R & Sarda N. (2005) Sleep wake profile and EEG spectral power in young or old senescence accelerated mice. Neurobiol. Aging, 26, 265-273.

[24] 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, 127-136.

[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, 8-19.

[26] Tobler I, Deboer T,& Fischer M. (1997) Sleep and sleep regulation in normal and prion protein-deficient mice. J. Neurosci., 17, 1869-1879.

[27] Franken P, Lopez-Molina L, Marcacci L, Schibler U & Tafti M. (2000) The transcription factor DBP affects circadian sleep consolidation and rhythmic EEG activity. J. Neurosci., 20, 617-625.

[28] Franken P, Malafosse A & Tafti M. (1999) Genetic determinants of sleep regulation in inbred mice. Sleep 22, 155-169.

[29] Steriade M, Contreras D, Curró Dossi R & Nuñez A. (1993b) The slow (< 1 Hz) oscillation in reticular thalamic and thalamocortical neurons: scenario of sleep rhythm generation in interacting thalamic and neocortical networks. J. Neurosci., 13, 3284-3299.

[30] Cefalu WT, Wang ZQ, Werbel S, Bell-Farrow A, Crouse JR, III, Hinson WH, Terry JG & Anderson R. (1995) Contribution of visceral fat mass to the insulin resistance of aging. Metabolism, 44, 954-959.

[31] Fujimoto WY, Bergstrom RW, Boyko EJ, Chen KW, Leonetti DL, Newell-Morris L, Shofer JB & Wahl PW. (1999) Visceral adiposity and incident coronary heart disease in Japanese-American men. The 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. Diabetes Care, 22, 1808-1812.

[32] Minamino T, Orimo M, Shimizu I, Kunieda T, Yokoyama M, Ito T, Nojima A, Nabetani A, Oike Y, Matsubara H, Ishikawa F & Komuro I. (2009) A crucial role for adipose tissue p53 in the regulation of insulin resistance. Nat. Med., 15, 1082-1087.

[33] Wienecke M, Werth E, Poryazova R, Baumann-Vogel H, Bassetti CL, Weller M, Waldvogel D, Storch A & Baumann CR. (2012) Progressive dopamine and hypocretin deficiencies in Parkinson's disease: is there an impact on sleep and wakefulness? J. Sleep Res., 21, 710-717.

[34] Slats D, Claassen JA, Verbeek MM & Overeem S. (2013) Reciprocal interactions between sleep, circadian rhythms and Alzheimer's disease: focus on the role of hypocretin and melatonin. Ageing Res. Rev., 12, 188-200.

[35] de Lecea, L. (2010) A decade of hypocretins: past, present and future of the neurobiology of arousal. Acta Physiol., 198, 203-208.

[36] Saper, C.B. (2013) The neurobiology of sleep. Continuum (Minneap. Minn.), 19, 19-31.

[37] Liblau, R.S., Vassalli, A., Seifinejad, A. & Tafti, M. (2015) Hypocretin (orexin) biology and the pathophysiology of narcolepsy with cataplexy. Lancet Neurol., 14, 318-328.

[38] Terao A, Apte-Deshpande A, Morairty S, Freund YR & Kilduff TS. (2002) Age-related decline in hypocretin (orexin) receptor 2 messenger RNA levels in the mouse brain. Neurosci. Lett., 332, 190-194.

[39] Nixon JP, Mavanji V, Butterick TA, Billington CJ, Kotz CM & Teske JA. (2015) Sleep disorders, obesity, and aging: the role of orexin. Ageing Res. Rev., 20, 63-73.

[40] Mackiewicz M, Nikonova EV, Zimmermann JE, Romer MA, Cater J, Galante RJ, Pack AI. (2006) Age-related changes in adenosine metabolic enzymes in sleep/wake regulatory areas of the brain. Neurobiol Aging, 27, 351-360.

[41] Barnea M, Madar Z, Froy O. (2009) High-fat diet delays and fasting advances the circadian expression of adiponectin signaling components in mouse liver. Endocrinology, 150, 161-168.

[42] Landolt HP. (2008) Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol. 75, 2070-2079.

[43] Oakes ND, Cooney GJ, Camilleri S, Chisholm DJ & Kraegen EW. (1997) Mechanisms of liver and muscle insulin resistance induced by chronic high-fat feeding. Diabetes, 46, 1768-1774.

[44] Tschop M & Heiman ML. (2001) Rodent obesity models: an overview. Exp. Clin. Endocrinol. Diabetes, 109, 307-319.

[45] Buettner R, Scholmerich J & Bollheimer LC. (2007) High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity, 15, 798-808.

[46] Damjanovic M & Barton M. (2008) Fat intake and cardiovascular response. Curr. Hypertens. Rep., 10, 25-31.

[47] Molteni R, Barnard RJ, Ying Z, Roberts CK & Gómez-Pinilla F. (2002) A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience, 112, 803-814.

[48] Frank MG. (2013) Astroglial regulation of sleep homeostasis. Curr. Opin. Neurobiol., 23, 812-818.

[49] Patil S & Chan C. (2005) Palmitic and stearic fatty acids induce Alzheimer-like hyperphosphorylation of tau in primary rat cortical neurons. Neurosci. Lett., 384, 288-293.

[50] Amzica F & Neckelmann D. (1999) Membrane capacitance of cortical neurons and glia during sleep oscillations and spike-wave seizures. J. Neurophysiol., 82, 2731-2746.

[51] Amzica F & Massimini M. (2002) Glial and neuronal interactions during slow wave and paroxysmal activities in the neocortex. Cereb. Cortex., 12, 1101-1113.

[52] Magistretti PJ. (2011) Neuron-glia metabolic coupling and plasticity. Exp. Physiol., 96, 407-410.

[53] Wisor JP, Rempe MJ, Schmidt MA, Moore ME & Clegern WC (2012) Sleep slow-wave activity regulates cerebral glycolytic metabolism. Cereb. Cortex, 23, 1978-1987.

[54] Dash MB, Tononi G & Cirelli C (2012) Extracellular levels of lactate, but not oxygen, reflect sleep homeostasis in the rat cerebral cortex. Sleep, 35, 909-919.

[55] Naylor E, Aillon DV, Barrett BS, Wilson GS, Johnson DA, Johnson DA, Harmon HP, Gabbert S & Petillo PA. (2012) Lactate as a biomarker for sleep. Sleep, 35, 1209-1222.

[56] Woods S. C., Seeley R. J., Rushing P. A., D'Alessio D.& Tso P. (2003) A controlled high-fat diet induces an obese syndrome in rats. J. Nutr., 133, 1081-1087.

[57] Karaouzene N, Merzouk H, Aribi M, Merzouk SA, Berrouiguet AY, Tessier C & Narce M. (2011) Effects of the association of aging and obesity on lipids, lipoproteins and oxidative stress biomarkers: a comparison of older with young men. Nutr. Metab. Cardiovasc. Dis., 21, 792-799.

[58] Bruce-Keller A. J., White C. L., Gupta S., Knight A. G., Pistell P. J., Ingram D. K., Morrison C. D. & Keller J. N. (2010) NOX activity in brain aging: Exacerbation by high fat diet. Free Radic. Biol. Med., 49, 22-30.

[59] Merat S, Fruebis J, Sutphin M, Silvestre M, Reaven PD. (2000) Effect of aging on aortic expression of the vascular cell adhesion molecule-1 and atherosclerosis in murine models of atherosclerosis. J Gerontol A Biol Sci Med Sci., 55, B85-B94.

[60] Cousins SW, Espinosa-Heidmann DG, Alexandridou A, Sall J, Dubovy S, Csaky K. (2002) The role of aging, high fat diet and blue light exposure in an experimental mouse model for basal laminar deposit formation. Exp Eye Res., 75, 543-553.

[61] Choi YS, Goto S, Ikeda I, Sugano M. (1989) Interaction of dietary protein, cholesterol and age on lipid metabolism of the rat. Br J Nutr., 61, 531-543.

[62] Engler MM, Engler MB, Nguyen H. (1998) Age-related changes in plasma and tissue fatty acid composition in Fischer 344 rats. Biochem Mol Biol Int., 46, 1117-1126.

[63] Russell SJ & Kahn CR. (2007) Endocrine regulation of ageing. Nat. Rev. Mol. Cell Biol. 8, 681-91. Review.

Supplementary Material

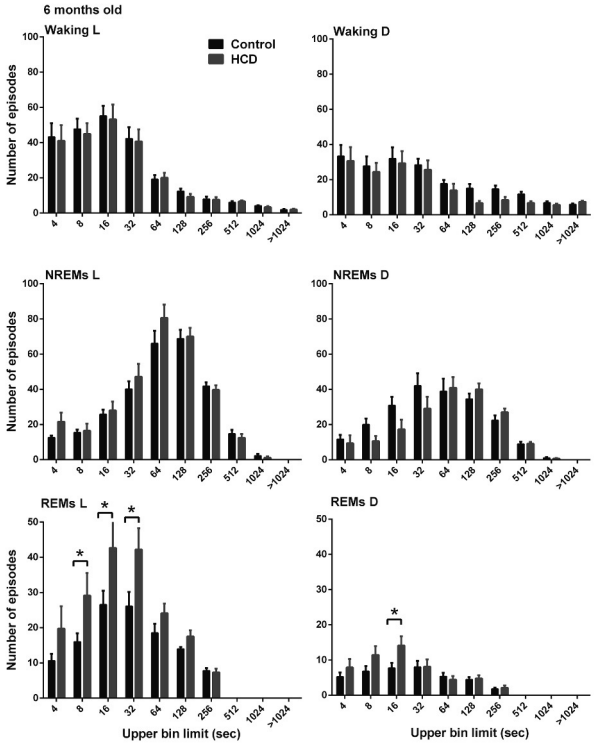

Figure 7.1: Histogram of vigilance state episodes frequency during the baseline light (L) and dark (D) period. Episodes were partitioned in ten exponentially increased duration bins (upper bin limit from 4 to >1024s) for young control high-caloric-diet (HCD) fed mice in baseline light and dark periods. 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', 'duration bin').

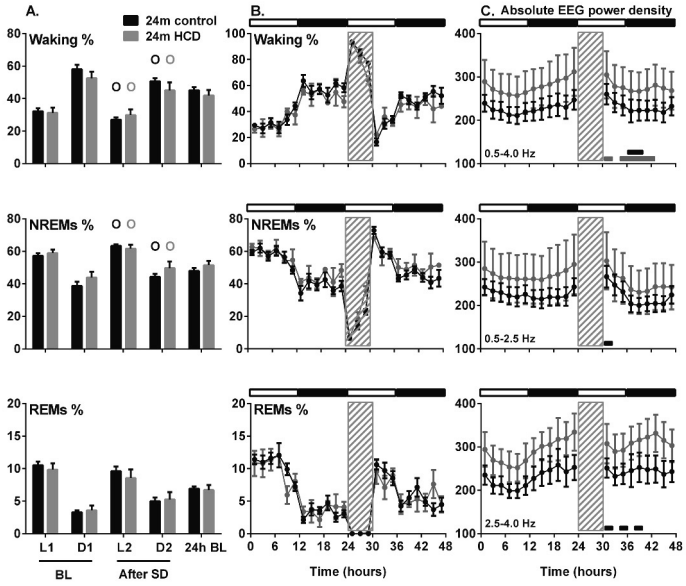

Figure 7.2: A. Light-dark distribution of each vigilance state (Waking, NREM and REM sleep) during the baseline day (BL: L1, D1) and after sleep deprivation (after SD: L2, D2). Bar plots represent mean ($\pm$ SEM) values (L1, D1, D2 correspond to 12-h values and L2 to 6-h values for the recovery period after SD, for light and dark 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 and high-caloric diet fed mice (HCD) (n=9 black bars and n=4 gray bars respectively). B. Time course of vigilance states (Waking, NREM sleep and REM sleep), for 24-h baseline (BL), 6-h sleep deprivation (SD, hatched bar) and 18-h recovery. Curves connect 2-h mean ($\pm$ SEM) values. The black and white bars above each graph indicate the light-dark cycle. No differences were found between groups (p>0.05). C. Time course of absolute electroencephalographic (EEG) power density ($\mu V^2/0.5Hz$) for the overall slow-wave activity range (SWA, 0.5-4 Hz) in non-rapid-eye movement (NREM) sleep, for the slow SWA 0.5-2.5 Hz and the fast SWA 2.5-4 Hz for 24-h baseline (BL), 6-h sleep deprivation (SD, hatched bar) and 18-h recovery. Black and grey bars represent differences for baseline and after sleep deprivation days.

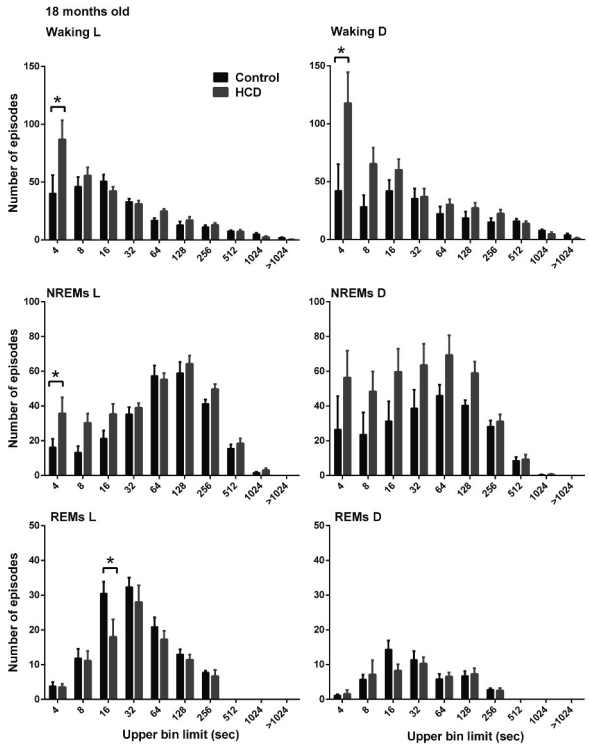

Figure 7.3: Histogram of vigilance state episodes frequency during the baseline light (L) and dark (D) period. Episodes were partitioned in ten exponentially increased duration bins (upper bin limit from 4 to >1024s) for 18 months old control high-caloric-diet (HCD) fed mice in baseline light and dark periods. 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', 'duration bin').

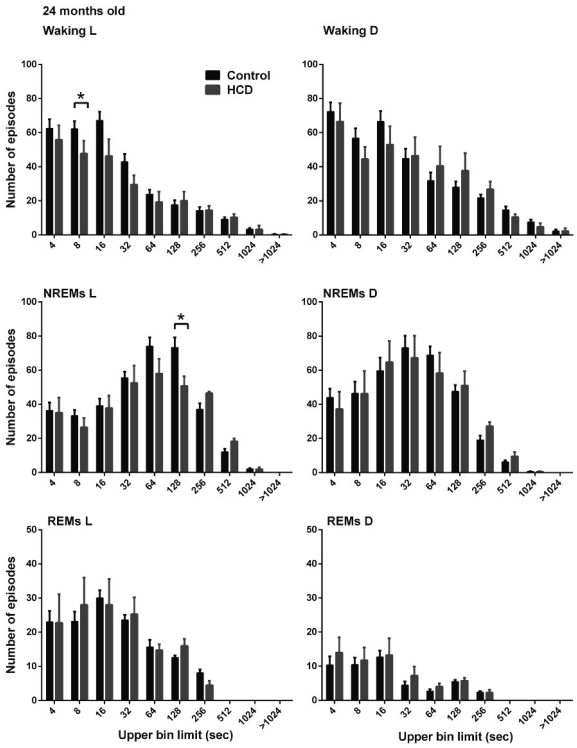

Figure 7.4: Histogram of vigilance state episodes frequency during the baseline light (L) and dark (D) period. Episodes were partitioned in ten exponentially increased duration bins (upper bin limit from 4 to >1024s) for 24 months old control high-caloric-diet (HCD) fed mice in baseline light and dark periods. 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', 'duration bin').

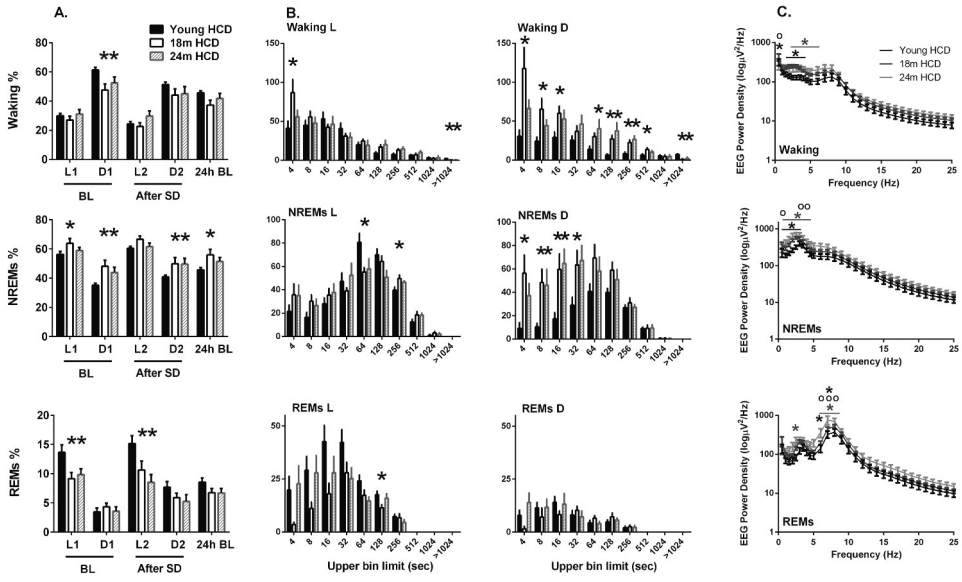

Figure 7.5: A. Light-dark distribution of each vigilance state (Waking, NREM and REM sleep) during the baseline day (BL: L1, D1) and after sleep deprivation (after SD: L2, D2). Bar plots represent mean ($\pm$ SEM) values (L1, D1, D2 correspond to 12-h values and L2 to 6-h values for the recovery period after SD, for light and dark periods during the 48-h recordings respectively) and 24-h values of baseline recordings (24-h BL) for Waking, NREM and REM sleep for all high-caloric-diet fed (HCD) groups. B. Histogram of vigilance state episodes frequency during the baseline light (L) and dark (D) period. Episodes were partitioned in ten exponentially increased duration bins (upper bin limit from 4 to >1024s) for young, 18 and 24 months high-caloric-diet (HCD) fed mice in baseline light and dark periods. Asterisks represent differences between groups (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'age', 'Light-Dark', 'day'). C. Electroencephalographic (EEG) power density in Waking, NREM and REM sleep for young, 18 months and 24 months old high-caloric-diet (HCD) fed mice (log $\mu V^2/Hz$, 0.5-25 Hz). Black and gray asterisks indicate significant differences of 18 months and 24 months old respectively compared to young HCD fed mice and black circles significant differences between 18 months and 24 months old HCD fed mice (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'age', 'EEG frequency bin').

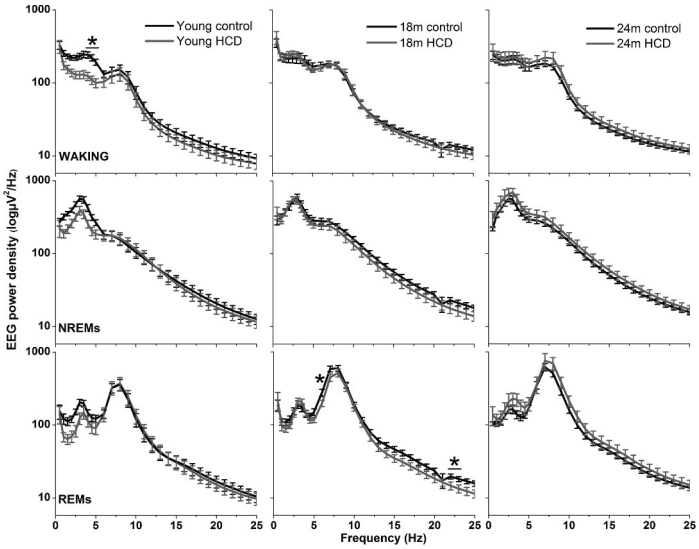

Figure 7.6: Electroencephalographic (EEG) power density in Waking, NREM and REM sleep for young, 18 months and 24 months old control and high-caloric-diet (HCD) fed mice (log $\mu V^2/Hz$, 0.5-25 Hz). Asterisks indicate significant differences between control and HCD fed groups (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'treatment', 'EEG frequency bin').

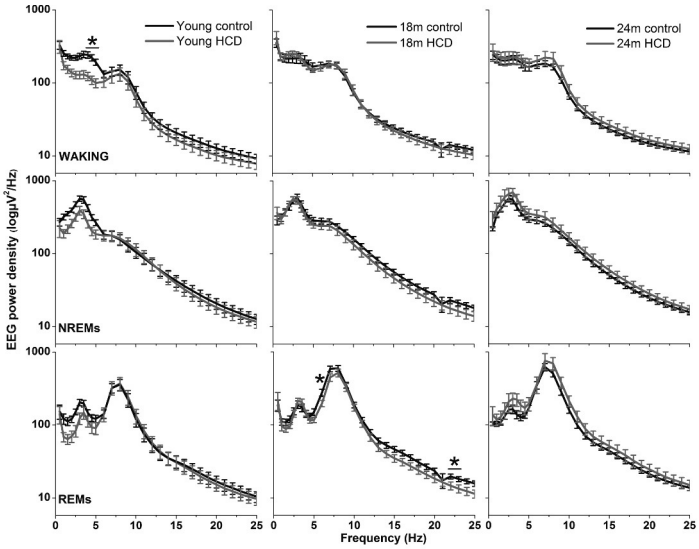

Figure 7.7: Electroencephalographic (EEG) power density in NREM sleep (expressed as %12-h values of the light period) in the frequencies between 0.5 and 25 Hz in 2-h time bins across the BL light period for young (green line), 18 months (dark gray line) and 24 months (light gray line) old high-caloric-diet (HCD) fed mice and young controls (black line). Black and gray asterisks indicate significant differences of 18 months and 24 months old HCD fed compared to young HCD fed mice respectively, black circles significant differences between 18 months and 24 months old HCD fed mice and black lines on the top of each graph indicate significant differences between young control and young HCD fed mice (post-hoc unpaired t-tests with Bonferroni multiple comparisons correction, $p<0.05$ after significant ANOVA, main effects 'age', 'EEG frequency bin', '2-h intervals').