Sleep alterations in the course of aging environmental inputs

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Chapter 10

General Discussion

During our life span, a great amount of time is spent in sleep. Although not fully conceived why, sleep is unambiguously an important process for the brain and the whole organism. In aging, in congruence with general health deterioration, rendering the organism more vulnerable to disease, sleep is also altered. Several environmental factors, to whom we are daily exposed, affect the sleep behavior from adulthood to aging. In this thesis, an attempt has been made in order to elucidate the way sleep and subsequently general health can be enhanced in the course of aging.

10.1 Sleep characteristics in the course of aging

In accordance with numerous alterations, beginning with biochemical changes at the molecular level, that eventually expand to encompass the cellular, tissue, and organ system level, aging is associated with sleep and circadian changes in humans and animals. **Chapter 5** is the first of the series of chapters in the current thesis which follows a basic but detailed approach on sleep effects in the course of aging in the mouse model C57BL/6J. In **Chapter 5** it is demonstrated that older mice displayed different sleep characteristics regarding sleep architecture and the sleep electroencephalogram (EEG) as compared to young mice. In particular, the older mice showed more non-rapid eye movement (NREM) sleep and less waking especially during the dark period, emerging from an increase in the number of long NREM sleep episodes and a decrease in the number of long waking episodes respectively, as well as less REM sleep at the end of the light period. Although the sleep features of older mice depicted in **Chapter 5** are in line with earlier studies on the same and other mouse strains, the associated mechanisms underlying the changes remain uncharted [1, 2, 3, 4, 5, 6]. As shown in Figure 2 of the introduction of this thesis (**Chapter 1**), sleep and wakefulness are regulated by different brain circuits in which monoaminergic neurons are the protagonists. Since alterations in neurotransmitters such as adenosine, hypocretin (orexin), glutamate, GABA and others or their receptor balance are likely to induce changes in the sleep-wake circuit, they have been proposed to play an important role in sleep changes seen in aging [7, 8].
A significant result obtained in **Chapter 5** is that sleep in older mice is characterized by increased EEG slow-wave activity (SWA, EEG power in 0.5-4.0 Hz) in NREM sleep and altered slow-wave morphology compared to young mice. This suggests that older mice possibly live under higher sleep pressure conditions. In addition to that, the slower decay rate in SWA in the first three hours after sleep deprivation in the older mice suggests a decreased efficiency in dissipating sleep pressure with age. The morphological slow-wave findings in the old mice including the altered slow-wave slopes, the decreased sigma activity (activity between 9-13 Hz) during the down state, and the decrease in the number of multipeak waves, point towards altered brain network properties, suggesting increased neuronal coupling at the local level between cortical neurons, at the expense of the global connectivity. Although increased slow-waves in NREM sleep were also found in older mice in a more recent study, validating our data, it was demonstrated that the local cortical neural dynamics and local sleep homeostatic mechanisms were not impaired during healthy senescence in mice [6].

Fragmented behavioral rest-activity patterns, as well as reduced amplitude of electrical activity rhythms in the suprachiasmatic nucleus (SCN) characterize old mice, indicating that aging substantially affects the circadian clock [9, 1]. Interestingly, behavioral activity alterations in mice arise at the age of 12 months [1] (Figure 10.1 A). Sleep alterations in the course of aging seem to follow a similar path (Figure 10.1 B,C). Characteristically, in figure 10.1, sleep parameters are compared between 6, 12, 18 and 24 months old mice that were sedentary their whole life: 12 months old mice show circadian period and locomotor activity levels similar to older mice, intermediate vigilance state characteristics between young and older mice and EEG SWA in NREM sleep similar to older mice, which significantly differs from young mice (unpublished data). Therefore, we can conclude that an aging phenotype in sleep and circadian rhythms is apparent relatively early in a mouse life, considering that 12 months corresponds to earlier than middle age in the mouse life span.

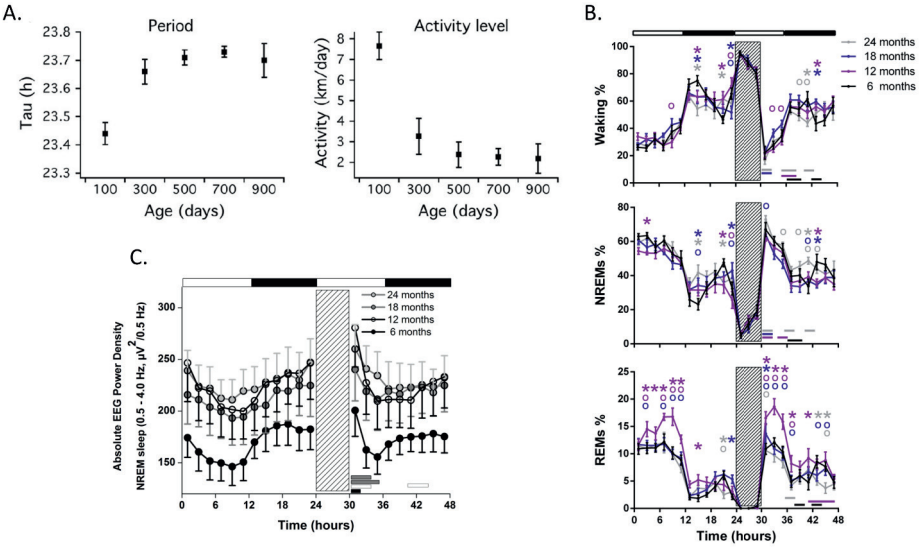

Figure 10.1: Sleep and behavioral alterations in the course of aging. A: Period and activity levels of mice at the age of 100, 300, 500, 700 and 900 days (Adapted from Farajnia et al., 2012 [1]), B: Time course of vigilance states for 48-h continuous sleep recordings in 6, 12, 18 and 24 months old mice without any environmental intervention, along with C: their EEG slow-wave activity in NREM sleep (see text for more details)

Interestingly, in contrast to the circadian clock changes, humans show different sleep patterns from mice as they grow older. In elderly humans, problems falling asleep, increased sleep fragmentation, decreased total sleep time and sleep efficiency, as well as attenuated slow-wave activity in NREM sleep (EEG SWA, power density between 0.5-4.0 Hz) are noted [10, 11, 12, 13]. The complete spectrum of age-related sleep alterations in aging was only recently fully documented, showing in most cases results in the opposite direc-

tion in laboratory mice as compared to humans. Nevertheless, these findings, including findings presented in **Chapter 5**, do not invalidate the mouse model as an important laboratory tool in sleep research. At the local cortical network level, there is a similarity between humans and laboratory animals since no evident alterations are apparent in the course of aging [6]. Markedly, disparities should be always taken into account when comparing between species, since considerable insights can be obtained. Animal models signify a necessity for the scientific world, however, it is shallow to consider that equal results will be obtained in humans in comparison to other animals. Through **Chapter 5**, therefore, it is established that in laboratory animals, in addition to attenuated behavioral activity, and altered amplitude and timing of circadian rhythms, sleep parameters are also affected as a function of age, albeit the mechanisms underlying these age-associated sleep changes remain elusive [2, 3, 1, 4, 5, 6].

The aging process, following the path from birth to the end of life, lasts for approximately two years in a mouse model, such as the C57BL/6J mouse strain. However, recent advances in technology and science warrant for successful shorter time-span studies. Thus, in addition to naturally aged mice, sleep parameters were investigated in a genetic mouse model of accelerated aging, portrayed in **Chapter 9**. In this chapter, we aimed at investigating a premature aging mouse model in order to assess its validity as a mouse model appropriate for sleep research. As detailed reported in **Chapter 1**, there are aging theories supporting the idea that active free radicals, normally produced in the organism, may induce aging by damaging the cellular components, ultimately leading to cell death and tissue dysfunction [14]. Since DNA comprises a major target for oxidants, DNA damage has to be restored through associated repair mechanisms. The latter are important mechanisms in the aging process, that, if overruled, could lead to premature aging and mortality [15]. An animal model based on the complete deficiency of DNA repair protein is the xeroderma pigmentosum Group G protein (XPG) knockout model [16]. Animals with complete deficiency of the XPG protein have a very short life span and progeria [17, 18]. Taking into account the significant properties of this mouse model for aging research, in **Chapter 9** we initially studied the sleep characteristics and subsequently compared them with data obtained in naturally aged mice.

Aged mutant mice shared similar sleep architectural characteristics to naturally aged mice, that differed from young mutants and age-matched wild type littermates (**Chapter 9**). These consist of less waking and more NREM sleep in the first half of the dark period and attenuated REM sleep during the light period. Despite these similarities with natural aging, the decreased amplitude of the 24-h vigilance state rhythm of the aged Xpg-/- mice led to more waking at the end of the light period and a lower overall REM sleep amount in the 12-h light period compared to 24 months old C57BL/6J mice, likely showing an exacerbated aging phenotype. In contrast to sleep architecture, EEG power density in all vigilance states across a wide frequency spectrum was found to be profoundly attenuated in young and aged Xpg mutant mice deviating from patterns seen in naturally aged mice. Since this was evident in all vigilance states, it can be suggested that sleep quality and brain integrity are likely compromised in the Xpg-/- mouse model even at the corresponding young adult age.

Notably, during the recordings, a torpor-like state was observed in the aged mutant mice, particularly at the end of the dark period, in which hypothermia was evident. The animals had low skin temperature and the EEG amplitude was markedly reduced. It is likely that

Xpg-/- mice show metabolic changes which may result in decreased body temperature and the subsequent occurrence of torpor bouts, in accordance with mice which are at the very end of their natural life [19, 20, 21, 22]. Concluding, although some similar sleep characteristics to naturally aged mice are apparent, XPG deficiency possibly alters physiological aging beyond what is usually found and therefore this premature aging mouse strain denotes a more extreme aging condition, not occurring under natural conditions.
Conversely to the first ideas in the neuroscience field developed some centuries ago, that the adult nervous system is rather hard-wired and non-resilient, the last decades it is established through laboratory animal and human studies, that the adult mammalian brain obtains plastic properties which can be continuously reformed by environmental input [23, 24]. In the two following sections we are discussing environmental inputs, commonplace in everyday life, that positively or negatively affect sleep and subsequently brain function during aging.

10.2 Factors that impair sleep and behavior

Aging is an unavoidable process that affects the development of organisms throughout the lifespan. Healthy aging comprises an utmost goal of every individual. However, an array of debilities emerges not only in pathological but also in healthy aging. As discussed earlier, sleep alterations are commonly viewed in aging. When the environment intervenes with aging, detrimental effects arise for general health that could consequently even accelerate or accentuate aging. **Chapters 2, 4, 7 and 8** aim at investigating sleep and circadian alterations following long-term high-caloric diet consumption and dim-light-at-night exposure.

10.2.1 High-caloric diet

Diet-induced obesity is a modern disease that affects big parts of the population worldwide. General health is compromised in people with obesity that can eventually lead to secondary diseases and ultimately to increased mortality even at a young age [25]. Obesity, in conjuction with physical inactivity, is likely to increase the risk for cardiovascular disease, Type 2 diabetes, hypertension, insulin resistance, neurodegeneration, dyslipidemia and certain cancers [26]. Additionally, obesity and metabolic disorder are accompanied by chronic low-grade inflammation, linked to alterations in brain function such as hypothalamic inflammation [27, 28, 29, 30]. One possible factor that may contribute to obesity is sleep behavior, owing to an increase in sleep debt due to modern lifestyle and working conditions [31, 32]. Concurrently, obesity directly or indirectly may induce sleep disturbances [33, 34]. Therefore, obesity and sleep are two interrelated entities that are investigated in **Chapters 2 and 7**.
In **Chapters 2 and 7** young adult (6 months old) and aged mice (18 months old) were fed exclusively with high-caloric diet (HCD) for three months, signs of obesity being evident even after the first week of this diet. An increase of almost 65% of the body weight was apparent following twelve weeks of treatment. In young HCD fed mice, moderate sleep architectural alterations were found compared to age-matched control mice, denoted by

an increased REM sleep amount emerging from an increased probability of consecutive NREM-REM sleep cycles. Instead of sleep fragmentation usually assumed in obese subjects, increased sleep consolidation was shown in the data, confirming earlier studies [35, 36, 37, 38]. Due to the increased REM sleep, and the close relationship between REM sleep and thermoregulation [39], we hypothesized that young mice fed chronically with high-caloric diet, would probably show an altered thermoregulatory activity along with modulated metabolic rate. Interestingly, recent findings on young HCD fed mice indicate that body temperature of obese mice is approximately 1 degree higher compared to lean mice, validating hence our hypothesis of a reduced thermoregulatory demand in the HCD fed mice [40].
It is known that aging leads to a redistribution of body fat with similar changes to the ones seen during HCD consumption [41, 42]. In addition, increased total fat mass and elevated levels of abdominal adipose tissue are noted at middle age, being characterized as risk factors for several diseases [41, 43]. Thus, the hypothesis was formed in **Chapter 7** that obesity and aging may act synergistically, negatively affecting sleep. The data demonstrated that sleep architecture was altered in 18-months old HCD treated mice. They showed increased NREM sleep and decreased waking, compared to age-matched controls, denoting a potentially enhanced aging phenotype in the sleep architecture.
In **Chapter 2** insights regarding the regulation of sleep were investigated, by applying parameter estimation analysis and mathematical modeling of the observed homeostatic sleep response. The observed homeostatic sleep response was visible in the EEG SWA, which is directly associated with the homeostatic sleep process. Young HCD fed mice in addition to an overall decrease in absolute EEG SWA during NREM sleep, showed a slower build-up of sleep pressure during waking and REM sleep. The data converge that HCD alters sleep homeostasis, rendering young mice less susceptible to prolonged waking. On the contrary, as it is shown in **Chapter 7**, older HCD fed mice showed increased levels of SWA in NREM sleep particularly in the slow component (0.5-2.5 Hz), as compared to both young HCD fed, young and aged controls. Although aging is the prevailing parameter, it seems that regarding SWA, obesity adds further effects in aged mice.
Besides the finding that functioning of the central clock remains intact following HCD [44], it seems that HCD, chronically consumed by young mice, provides short-term benefits by attenuating the burden of the sleep debt, since it reduces the effect of prolonged waking on subsequent EEG SWA in NREM sleep. Although, at first glance, the data seem to point against adverse effects of HCD on sleep and the circadian clock, particularly during young adulthood, the persistent increased weight, already associated with a multitude of diseases, along with aging is demonstrated to be remarkably detrimental for sleep, and general body and brain health in older animals.

10.2.2 Dim light at night

In modern society, in addition to dietary preferences and food availability, including ultra-processed and junk food that could eventually lead to obesity, there is a widespread use of artificial light which is also associated with various health disturbances. Although the invention of electrical light accounts for numerous advantages nowadays, light expo-

sure particularly at night has been linked with metabolic, immunological, and behavioral rhythm disturbances across a wide age spectrum [45]. In **Chapters 4 and 8**, we study the effects of dim light at night (DLAN) exposure on sleep, the sleep EEG and circadian behavior in young and aged mice.
The data showed that, following merely one night of DLAN exposure, there are altered sleep parameters, including a delay of vigilance state rhythms which increases as a function of DLAN exposure from one day to one month (**Chapter 4**). This is in accordance to earlier findings in rats [46] as well as temperature rhythm findings found to be accordingly delayed in lemurs [47]. After three continuous months of DLAN exposure, severe effects on sleep and rest-activity rhythms were evident in young mice, with the dynamics of the daily light-dark/dim amplitude being significantly distorted, similarly to aged mice exposed to DLAN (**Chapters 8**). Additional analysis on the rest-activity data of young mice revealed that chronic DLAN attenuates daily fluctuations corresponding to healthy physiology and negatively affects brain network's integrity [48].
Interestingly, the most pronounced changes in the sleep EEG were found in young mice, demonstrating a general power attenuation across all prominent frequencies (**Chapters 4 and 8**). In accordance with the diminished rhythmicity in rest-activity after three months of DLAN exposure, the spectral alterations suggested a reduced quality of brain cortical activity in all three vigilance states. The general decrease in EEG power density may be caused by a loss of thalamo-cortical synchronization due to prolonged DLAN exposure [49]. Nevertheless, the cerebral cortex is not just a passive receiver of synchronized delta potentials of thalamic origin, but these inputs are reorganized by the intrinsic properties and synaptic events in cortical circuits, therefore, alterations following DLAN exposure may also take place at the cortical level [49, 50]. After chronic DLAN exposure, EEG SWA in NREM sleep was differentially affected in 6, 18 and 24 months old mice, showing an attenuation in the young mice, and an increase in the 24 months old mice while no alteration was found in the 18 months old mice. Therefore, long-term DLAN exposure induces pernicious sleep and circadian effects in young and aged mice alike, impacting first the sleep regulatory system and brain integrity in young mice and second accentuating age-induced sleep characteristics in aged mice.
Aging is an inevitable process which can be accelerated or decelerated depending on our lifestyle choices. By opting to abstain or lessen exposure to agents detrimental for general health, the path towards longevity could be ensured. Because what determines normal longevity, is the temporal aspect and the actual quality of the process of aging. Diet and light constitute two considerable environmental factors in everyday life. **Chapters 2, 4, 7 and 8** discussed in this section are, therefore, dedicated to the investigation of the effects on behavior & sleep following unhealthy diet, such as HCD consumption, and light exposure, particularly DLAN. Through these chapters it is indicated that HCD and DLAN largely impact sleep and circadian behavior, the sleep EEG as well as the underlying network during adulthood as well as aging.

10.3 Factors that enhance sleep and behavior

It is widely known that, in the course of aging, several body and brain functions gradually decline irreversibly. Contrary to environmental factors that impair sleep and its function,

impeding eventually general health during aging, other factors induce antithetical effects. In **Chapter 6** of the present thesis, the effect of long-term physical activity is investigated on sleep, the sleep EEG and behavior, through a running-wheel available in the cages of young and aged mice.

10.3.1 Physical activity

Exercise is generally considered beneficial for health across all ages [51, 53, 54, 55]. During the last decades, a large body of literature has focused on benefits of aerobic exercise, on cognitive and executive function in children, whose brains are highly plastic [56, 58, 57]. Another sensitive age group that could value from external but not invasive tools are the elderly. Epidemiological studies have shown the advantageous relationship of physical exercise and cognition in pathological and normal aging, exerting beneficial effects on memory function as well as protecting from age-related cognitive decline [59, 60, 61, 62, 63]. An abstinence of sedentarism through a more active lifestyle that includes physical activity, has been demonstrated to improve cardiovascular health, enhance stress reduction, ameliorate or prevent depression and anxiety [51]. The mechanisms underlying the main effects of physical activity suggest neurogenesis, neurotrophic factors, angiogenesis and improvement of mood [52]. As far as sleep is concerned, exercise has been proposed as an alternative treatment in order to ameliorate potential sleep disturbances in both young and aged subjects [64, 65, 62, 66]. In order to assess whether sleep parameters in aging, elaborately depicted in **Chapter 5** can be counteracted, **Chapter 6** is dedicated to the long-term effects of exercise on sleep, since analogous studies in humans and laboratory animals are scarce.

Young and aged mice, used in this study, were provided with a running wheel in their cages, for voluntary use on a daily basis for one to three months. Since running wheels were permanently removed two weeks prior to the sleep recordings, the sustained effects of exercise were studied. Young mice provided with a wheel were more awake and slept less in the dark period compared to young controls. These effects resembled the results seen in mice that are concomitantly recorded with a running wheel [67, 68] showing that a sustained exercise effect exists even after the two weeks of wheel removal. In the course of aging, this effect was attenuated, mildly affecting the sleep architecture of 18 and 24 months old mice. Equivalently, biomarkers of brain activity and neurogenesis are altered owing to exercise in mice across all age groups studied, albeit only young animals are able to maintain a significant increase above baseline levels, after two or four weeks after the end of exposure to a running wheel [69, 70, 71].

The age-related increase apparent in the course of aging in EEG SWA levels, was found to be counteracted in **Chapter 6**. In particular, it was found that long-term physical activity diminished the age-associated EEG SWA towards levels similar to young sedentary mice (Figure 4 of **Chapter 6**). In contrast to sleep architecture, this effect of long-term voluntary exercise on EEG SWA was sustained for at least two weeks after removal of the running wheel across all ages. This shows that introduction of moderate activity even later in life induces significant changes towards a younger brain phenotype. Due to the revealing features of EEG SWA in all age groups, we conducted additional analysis using information-theoretic and machine-learning approaches and found that characteristic

information regarding age and exercise was enclosed in SWA. Furthermore, with cluster analysis, we could classify and accurately distinguish the different groups based solely on their SWA. Taking into account recent findings regarding local cortical neural dynamics as well as local sleep homeostatic mechanisms which probably remain unaffected in aging [6], our study suggests that global dynamics associated with age-induced alterations are mainly affected through regular physical activity leading to a potential 'younger phenotype' (**Chapter 6**).
In humans, shrinkage of medial temporal lobe structures has been found in middle-aged adults and older people [72, 73]. Hippocampal volume loss in particular, seen in aged humans, was found to be restored following one-year walking training [74]. Additionally, similar to our study, after short- and long-term exercise, SWA levels and slow-wave-sleep was altered in old humans, reaching levels closer to younger subjects [75, 76, 77, 64]. Concluding, age-matched, voluntary physical activity can lead to a 'younger phenotype', likely through delaying shrinkage, or even restoring the structure of various brain structures.
Aging inescapably occurs as a function of time in the organisms, in an asynchronous way in different brain areas, being characterized by a reduction in the reparative and regenerative potential in tissues and organs. The rate of the aging process is modulated by environmental factors and related to the neuronal-synaptic-molecular substrates of each area [8]. Henry David Thoreau writes about the time that flows *"Time is but the stream I go a-fishing in. I drink at it; but while I drink I see the sandy bottom and detect how shallow it is. Its thin current slides away, but eternity remains. I would drink deeper; fish in the sky, whose bottom is pebbly with stars."* Although we cannot overpass or overcome time and its outcome, life enrichment in the elderly, similar to environmental enrichment in animals, may comprise a key towards improvement of the quality of life sometimes lacking in really advanced age. Enrichment potentiates social interactions as well as learning, memory, sensory and motor stimulation, promoting plastic changes in the brain, known to be degraded in the course of aging [78, 79]. Our study in **Chapter 6** suggests that voluntary, long-term, age-matched exercise could attenuate the effects of sedentary behaviors and it could be prescribed as a first-order "medication" for general body as well as brain health augmentation throughout the whole age spectrum.

10.3.2 Caffeine

Caffeine is a xanthine alkaloid which is found in a variety of foods and beverages, such as chocolate, coffee, and tea. Coffee is the most commonly consumed drink after water worldwide [80]. Traditionally, it is believed that short- or long-term caffeine consumption disrupts sleep and may even induce health problems such as anxiety and increased stress, overwhelming fatigue, digestive disturbances, palpitations, high blood pressure and depression [81, 82, 83, 84]. Research has shown that caffeine ingested before sleep time may impact sleep by reducing the total sleep time and prolonging the sleep latency, with effects evident even with caffeine consumed in the morning [93]. Although a maximum daily intake of caffeine is recommended especially for children, adolescents and pregnant women, caffeine can have positive effects, increasing alertness and energy, well-being, relaxation, good mood and improved memory [91, 92, 83]. More particularly, caffeine has been used to treat apnea of prematurity, as a fatigue countermeasure by

truck drivers, shift workers, airline pilots and it is found in several medications against headache and appetite suppression [85, 86, 87, 88, 89, 90].

In **Chapter 3** we investigated the effects of acute and prolonged caffeine consumption on sleep and circadian parameters, by introducing caffeinated drinking water in the cages of the mice for up to two weeks. Validating the common belief that caffeine promotes arousal, the study confirms earlier findings that acute intake of caffeine increases waking with the concomitant diminished NREM sleep consolidation during the active phase [94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106]. While effects similar to the acute condition were found during the dark active period, following prolonged caffeine consumption, additional effects were found during the main sleep period with increased and deeper sleep. Interestingly, these effects were rather notable since caffeine has generally been considered to disturb sleep [107, 108]. A recent study supports adaptation following several days of caffeine intake in humans, showing that caffeine does not considerably shift melatonin and cortisol while it does not lead to an increased wake-promotion in the evening [109]. In accordance with spectral changes seen in mice provided with a running-wheel showing enhanced alertness, after chronic caffeine ingestion, the activity in the slow-wave range was markedly reduced while theta activity was accentuated [110, 111] suggesting that during waking, the animals were more active. Moreover, increased SWA was noted at the beginning of the light period, which together with the finding that NREM sleep duration was also increased during this period, suggests that sleep pressure is augmented. Sleep deprivation, additionally performed in this study, proved to be extremely challenging during chronic caffeine intake validating the notion of an increased sleep pressure in the first half of the light period.

It has been demonstrated that caffeine not only modulates sleep homeostatic mechanisms, but it also influences the circadian clock function [112, 113, 114]. Particularly, caffeine has been shown to increase circadian amplitude in vitro [115], while in addition it increases the influence of light on the SCN [114], which in turn may also lead to an increased circadian amplitude. Through **Chapter 3**, it is therefore suggested that an increase in the amplitude of the circadian clock, similar to the wheel access effect on the clock [116], likely underlies the increase in the 24-hour amplitude of the rest-activity and sleep-wake behavior, explaining this way the increase in SWA and sleep pressure at the beginning of the light period.

Chronic caffeine consumption has been linked with cognitive decline prevention, reduced risk of developing stroke, Parkinson's and Alzheimer's disease [117, 118, 119, 120, 121]. Since, in addition, caffeine amplifies cognitive performance, with an improvement in reaction time, vigilance and logical reasoning during extended periods with restricted opportunities for sleep [122], it can be suggested that daily caffeine intake can be part of a healthy balanced diet and its consumption does not need to be stopped in elderly people. Thus, in accordance with factors that enhance sleep and behavior such as the aforementioned effects of exercise, caffeine conversely to the traditional view may actually aid sensitive groups as for example the elderly by accentuating performance, alertness, increasing circadian amplitude and generally ameliorating sleep health. Future studies are needed in order to endorse this hypothesis for the elderly.

10.4 Biomarkers of brain age: inside EEG SWA in NREM sleep

Physiological aging is a complicated process whose molecular hallmarks and organ-specific physiological functions are affected by genetic, epigenetic, as well as environmental factors. In the current thesis, we focus on the environment and its effects on sleep and show that aging can be accentuated, accelerated or decelerated by the daily and long-term exposure to agents such as diet, physical activity and light, disentangling consequently the 'chronological' from the 'biological' age conceptions. Chronological age provides limited information regarding the complex processes that regulate aging. As a result, individuals with the same chronological age may vary in health, disease and disability, and therefore although coeval, they may differ in biological age [123, 124, 125]. Biological age does not perfectly correlate with time, since it is influenced by additional parameters as for example genetic background, disease, lifestyle. It has been demonstrated that even subjective perception of aging does not coincide with the chronological age and is associated with the process of brain aging and brain health, since feeling subjectively older likely reflects relatively faster aging brain structures [126, 127]. Therefore, the difference between chronological and biological or brain age may show advanced or delayed brain aging.

Biological information, which can be used to predict the brain age of an individual, has the potential to offer clinically relevant biomarkers, particularly useful for the elderly and the diseases associated with advanced age. The large neuroimaging datasets, in conjunction with newly developed machine learning techniques, have facilitated research regarding the estimation of brain age and biomarkers [128, 129, 130]. During the last decade, estimated brain age, primarily with the use of magnetic resonance image data, has been demonstrated to predict indicators of neurobiological aging, including cognitive impairment, obesity as well as diabetes [131, 132, 133, 135, 136]. Anatomical characteristics such as cortical thickness have also been used to successfully predict brain age, with a future application of an early prediction of neurocognitive disorders [137]. In addition to these features, brain EEG signals have been recently used in brain age prediction, since EEG traits, as for example EEG rhythms, alter as a function of age [138]. Age prediction from EEG has been studied with increased accuracy, using functional connectivity features from EEG resting-state recordings in order to correctly classify young and middle-age groups [139].

In addition to the aforementioned EEG attributes, sleep EEG can also provide elaborate information that could predict brain age, since significant changes are noted in it in the course of aging. Throughout the chapters of the present thesis, a common conjecture is the long-term effect of environmental factors on EEG SWA in NREM sleep. Depending on the positive or negative factor for general and brain health, effects on EEG SWA follow a specific direction in aged mice. In **Chapter 6**, we investigate the effect of long-term physical activity, a well-known advantageous agent that enhances general health, on sleep and the sleep EEG. We show that EEG SWA increases with age and attenuates with exercise, effects that are sustained even following some weeks after the cessation of the activity. Subsequently, taken into account these cross-age outcomes, we examine in this chapter whether EEG SWA contains information regarding the brain age of the mice. Thus, using machine learning techniques, we validate the hypothesis that characteristic

information concerning age and exercise is enclosed in SWA, enabling us to classify as well as successfully identify the different age groups based merely on their EEG SWA. Further studies in the present thesis (**Chapters 7 and 8**), exploring the deleterious effects of chronic high-caloric diet, including high-fat and high-sugar consumption, as well as continuous dim-light at night exposure on general and brain health, show that EEG SWA tends to follow the opposite direction compared to the results found following exercise. We propose, hence, that information derived from the sleep EEG, and particularly the EEG SWA, can be used as a biomarker of brain age in an analogous way to the studies depicted earlier. In accordance, recent studies confirm our considerations in relation to the biological information that can be obtained through the sleep EEG in general and the EEG SWA in particular which can be used to predict the brain age of an individual [140, 141].
EEG SWA is a marker of sleep need and sleep depth, and is profoundly affected by aging as it is shown in **Chapter 5** [142, 143, 6]. From a neuronal point of view, SWA reflects the dynamics of neuronal firing and more specifically, the ability of cortical neuron populations to engage in synchronized activity through synaptic connections [144]. Brain aging is generally influenced by progressive and regressive neuronal processes due to cell growth and death [145, 132]. In addition, environmental factors and health conditions of individuals, are likely to affect structural changes in the brain [146, 147, 148, 137]. Through biomarkers such as the EEG SWA, reflecting not only sleep-related changes but also functional brain changes, we could predict, identify, and eventually rectify the brain age so that general health is improved in aged as well as young individuals.

10.5 Concluding Remarks and Future Directions

The present thesis consists of studies dedicated in the investigation of environment and sleep in young adult and aged mice. The first part, including **Chapters 2, 3, 4**, delves into the long-term effects of high-caloric diet, both the acute and long-term effects of dim-light at night exposure as well as acute and prolonged caffeine intake on sleep and circadian parameters in young adult mice. The second part, consisting of **Chapters 5-9**, explores sleep and behavior in naturally and genetically modified aged mice in normal dietary, 12:12h light-dark, sedentary conditions as well as following prolonged high-caloric diet consumption, dim-light at night exposure and physical activity.
Throughout the results presented in the current thesis, the effects of environmental factors, aging as well as their combination on sleep, on circadian behavior and particularly EEG SWA provide contributions to sleep research. Effects are especially pronounced in young mice, showing that they are more susceptible to environmental changes, whereas, although present, effects on aged mice are overall more moderate. Long-term DLAN exposure in young mice is found to profoundly disrupt sleep and circadian behavior with characteristics similar to the ones found in aged mice (**Chapter 4**). Notably, a general EEG power attenuation in the prominent frequencies in all vigilance states likely reflects a widespread degradation in connectivity in EEG generating brain areas. In addition, chronic consumption of high-caloric diet led to an altered sleep regulation of young mice, showing short-term benefits in the case of sleep dept but being markedly detrimental on the long-term (**Chapter 2**). Following prolonged caffeine consumption and physical ac-

tivity, sleep and circadian behavior are positively substantially and similarly affected, with effects of exercise lasting for at least two weeks after activity cessation (**Chapters 3 and 6**). Although sleep and circadian behavior is mildly affected in aged mice as portrayed in the second part of the present thesis, the effects found particularly on the sleep EEG and the SWA suggest that aging in combination with chronic negative factors as dim-light at night and high-caloric diet act synergistically, accentuating age-related sleep effects with an impact on general and brain health, while physical activity in aged mice, comprising a positive lifestyle intervention, points towards a younger sleep phenotype promoting as a result body and brain health (**Chapters 6, 7 and 8**).

Nowadays, brain age prediction is an active research area, where brain age can be used in order to identify potential protective or deleterious factors for brain health as people age. Novel evidence suggests that an 'older'-appearing brain is linked to advanced physiological but also cognitive aging increasing concomitantly the risk of mortality [129]. According to the results of the present thesis, EEG SWA can be considered as a biomarker of brain age, marking distinct aging signatures that reflect beneficial or detrimental effects on body and brain health, due to specific lifestyle choices. Although owing to the underlying multisystem nature of the aging process, no single biomarker is likely to suffice, future studies should direct their attention towards a more integrative measurement, incorporating overall molecular biomarkers, physiological functional parameters as well as brain aging markers such as the one that we propose, the EEG SWA [125]. Longevity and healthy aging are not apparently synonymous but they should dovetail with each other. Taking into account the biomarkers that predict biological and brain aging, interventions promoting health even in advanced age can be indicated so that senescent changes and diseases' onset can be postponed or reversed and a life including fitness, vitality and time free of morbidity can be established [149]. In conclusion, although the discovery of the fountain of youth remains elusive, through the present thesis, specific lifestyles are recommended for successful aging and enhanced general, brain, body and sleep health.

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