Sleep alterations in the course of aging environmental inputs

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Appendices

Appendix A

A.1 Summary

By approaching the age of ninety, we will have spent approximately thirty years of our time sleeping; considering that, sleep must play a physiologically vital role in life irrespective of age. Although markedly puzzling and of enigmatic nature, both sleep and aging have been the subject of study for centuries, since almost all animals on the planet experience them. Several theories revolve around their existence and evolution converging on one point: along with several physiological processes that deteriorate in the course of aging, sleep is largely affected. In particular, stemming possibly from interrupted brain pathways and brain atrophy, sleep quality is considered to be progressively decreased in humans, due to attenuated capacity to initiate and/or maintain sleep. Sleep in the elderly includes decreased total sleep time and sleep efficiency, increased sleep fragmentation accompanied by problems falling asleep, and attenuated deeper sleep. Deeper sleep is reflected in the slow-wave activity (SWA) in the non-rapid eye movement (NREM) sleep electroencephalogram (EEG power in NREM sleep in 0.5-4 Hz), which in the elderly is found to be lower. In addition to sleep, the circadian clock, located in the suprachiasmatic nucleus of the hypothalamus, which normally controls rhythms in behavior and physiology with a period length of almost 24h, is also influenced by aging, where a reduction in the amplitude of its signal has been noted. A plethora of environmental factors, to which we are daily exposed, influence the sleep and circadian behavior from adulthood to aging. Since aging is an unavoidable process, an utmost goal of every individual is to achieve healthy aging with an augmented general body and brain health. The current thesis aims to investigate the way sleep and subsequently general health can be enhanced in the course of aging, exploring the detrimental and beneficial aspects of specific environmental factors, namely *diet, physical activity, light levels and caffeine intake*.

In order to establish the basis of how aging shapes the sleep architecture and sleep EEG in mice, we conducted an extensive study depicted in **Chapter 5**. Older mice were found to sleep more during the dark period compared to young ones, and antithetically to what it is shown in humans, SWA levels in NREM sleep were elevated. This study was particularly focused on the attributes of EEG slow waves, that were characterized by increased amplitude, steeper slopes and fewer multipeak waves. Our study suggests first, that aged mice are living under high sleep pressure conditions and, second, that altered brain network properties possibly prevail in aging.

Albeit the notable findings in **Chapter 5**, the mechanisms underlying these age-associated sleep changes remain elusive. Due to the complexity of the aging process, the development of a concrete and solid animal model is essential; however, in the sleep research field, in general, aging has not been profoundly studied since it comprises a strenuous and time consuming area. A need, therefore, has been developed for successful shorter timespan studies. Owing to that, in **Chapter 9**, we aimed to investigate sleep and the sleep EEG in a premature aging mouse model with a complete deficiency of the xeroderma pigmentosum group G protein (XPG). Animals with complete XPG deficiency usually have a very short life span (with a maximum life expectancy of 18 weeks) and display segmental progeria. Sleep architectural characteristics were found to be analogous to normally aged mice. Nevertheless, the sleep quality and brain integrity was likely compromised in this mutant model beyond normal aging, revealing an exacerbated aging condition usually not seen in naturally aged mice. The data point towards a different

sleep regulation in XPG deficient mice that awaits to be further studied.
One of the environmental factors directly linked with general health and longevity is diet. **Chapters 2 and 7** are dedicated in studying the effect of diet-induced obesity on sleep and its regulation in young adult and aged mice. In order to simulate the human condition, mice (6 and 18 months old) were fed for twelve weeks exclusively with high-caloric diet. Following chronic high-caloric diet, sleep architecture was moderately affected in young mice, including an increase in REM sleep during the light period, while regarding alterations in the EEG, lower NREM sleep SWA was found compared to controls. By applying mathematical modeling, an altered sleep homeostasis was demonstrated to be induced by high-caloric diet, establishing a bi-directional relationship between obesity and sleep. In contrast to young as discussed in **Chapter 2**, in aged mice **Chapter 7**, high-caloric diet had a significant impact on sleep architecture and sleep EEG. Particularly, more NREM sleep and less waking was found in both light and dark periods in high-caloric diet mice compared to age-matched controls, as well as an additional increase in the slow component of EEG SWA (0.5-2.5 Hz). Although aging is the dominant variable, the data denote a synergistic effect of aging and diet leading to the notion that chronically consumed high-caloric diet accentuates age-induced sleep changes.
A considerable factor, introduced in the previous century, is artificial light; albeit significant, it has been associated with drawbacks in health especially in humans when exposed during darkness. Dim-light-at-night, and its effects on sleep and the circadian clock in young and aged mice (18 and 24 months old) is the subject of discussion of **Chapters 4 and 8**. In **Chapter 4**, young mice were exposed to different duration periods of dim-light-at-night ranging from one day to three months. Sleep was already affected following merely one night of dim-light-at-night exposure, including a delay of vigilance state rhythms that increased as a function of dim-light-at-night exposure from one day to one month. Remarkably pronounced effects on sleep, rest-activity rhythms and the sleep EEG were noted following three continuous months of dim-light-at-night exposure, simulating data obtained from aged mice (**Chapter 8**), showing overall a sleep network deterioration. By conducting, in addition, a more elaborate mathematical analysis on the locomotor activity data, a less healthy rest-activity pattern was revealed in the long-term condition in young mice. In contrast to the young animals, EEG SWA in aged mice was differentially affected by long-term dim-light-at-night, as described in **Chapter 8**. Although a decrease was found in young mice, an increase in EEG SWA was shown in 24 months old mice while no alteration in the 18 months old compared to age-matched controls. Our results indicate that behavioral rhythmicity, sleep and brain integrity are compromised after chronic dim-light-at-night exposure in both young and age mice alike.
In contrast to factors that impair sleep and impede subsequently general health in the course of aging, other agents may cause opposite effects. An agent, generally considered to be beneficial for health across all ages is physical activity, which is studied in **Chapter 6**. Young (6 months old) and aged mice (18 and 24 months old) were provided with a running wheel in their cages for voluntary use on a daily basis for one to three months. The wheels were removed two weeks prior to the sleep recordings, therefore, a sustained effect of exercise was investigated. Sleep architecture was significantly affected by long-term exercise with the findings including an increased wakefulness during the dark period in young mice and mild alterations in the aged ones. Notably, long-term

exercise significantly counteracted the age-induced increase in SWA levels found in aging, as described in **Chapter 5**. Elaborating, it was found that long-term physical activity diminished the age-associated EEG SWA in levels very similar to young sedentary mice. By additionally performing machine learning approaches, we found that characteristic information regarding age and exercise was enclosed in the SWA data. Our data show that introduction of voluntary, long-term, age-matched exercise even later in life is able to bring about alterations towards a 'younger brain phenotype'. Through this study, we suggest that EEG SWA can be used as a biomarker of brain age and that physical activity could potentially be prescribed as a first-order 'medication' for body and brain health, throughout the whole age spectrum.

Another environmental factor, widely used in a daily basis, is caffeine whose intake effects on sleep behavior and the sleep EEG were investigated in young mice as described in **Chapter 3**. As a psychostimulant substance, when acutely consumed (i.e. one day) in the drinking water, it induced an increase in waking during the active phase and less deep sleep in the following rest phase, validating the common conjecture. In contrast, when caffeine in the drinking water was chronically consumed (for two weeks continuously), an increase in waking during the active period was found accompanied by an increase in sleep in the rest period. Additionally, EEG SWA in NREM sleep was markedly increased in the chronic condition compared to both the acute and control ones. The data suggest that, opposing the traditional conception on the impact on sleep, habitual caffeine consumption has effects on sleep and sleep regulation potentially providing a therapeutic window.

The current thesis investigates the effects of environmental inputs on sleep, circadian behavior and the sleep EEG in young and aged mice. Our data show that, effects are especially pronounced in young mice, denoting an increased environmental susceptibility, while, albeit present, they are more moderate in older mice. According to the results narrated in the present thesis, EEG SWA can be considered as a biomarker of brain age, marking well-defined aging signatures that reflect deleterious or advantageous effects on body and brain health owing to specific lifestyle choices. Concluding, although the elixir of youth has not been discovered yet, our data provide significant insights into environmental agents that intervene in the aging process and may allow for a healthier and longer life span.