Sleep alterations in the course of aging environmental inputs

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Chapter 1

General introduction and aim of the project

1.1 Sleep function and mechanisms, sleep homeostasis

1.1.1 Sleep function

In Greek mythology, *Hypnos* ("sleep") was considered to be a god or a demon that comprised the personification of sleep. According to Hesiod, Hypnos had a twin brother named *Thanatos* ("death"), both being sons of *Nyx* ("The Night") and *Erebus* ("The Darkness"). Hypnos was thought to live in a cave, at the entrance of which grow a number of poppies and other hypnotic plants, where the river *Lethe* ("Forgetfulness") comes from and where night and day meet. No light and no sound would ever enter his grotto [1].

Aristotle argued that sleep arises from the concentrated hot matter (moist and solid) exhaled from ingested food, and that in the course of sleep, the organ of sense perception should lose its power after its use, so that no animal perpetually actualizes its powers. From Aristotle's definition, our understanding of sleep is much evolved, however, it is still not fully conceived. Sleep resembles a death or coma-like state, but differs from it as it is easily reversible. Until the 1950's, sleep was considered a passive, dormant part of daily life, during which brain activity was almost absent [2]. Scientists such as Pavlov and Sherrington abided by this viewpoint [3, 4]. These assumptions were debunked by the discovery of regular cyclic alterations of rapid eye movement and non-rapid eye movement sleep phases, which was a springboard to the belief that sleep is actually an actively regulated process [2, 5]. Following decades of scientific work, sleep can be defined now as an easily reversed state of reduced responsiveness, associated with a particular immobile posture and a specific sleeping site, during which the brain is highly active [6].
Almost all animals on the planet sleep, nevertheless, whether sleep is essential remains an enigma [7]. Several theories have been proposed in the attempt to elucidate the function of sleep [8, 9, 10]. *First*, it has been proposed that sleep serves energy conservation [11, 12]. Rather like recharging a battery, it is proposed in this theory that during sleep, energy resources can be conserved for a considerable amount of time, so that survival is ensured when food is scarce. Energy savings may have been involved in the sleep process and selection during evolution. The findings that sleep cycle length and sleep amounts are negatively correlated with body and brain size, with smaller animals having higher metabolic rates, smaller body size and longer sleep, further support this theory [11, 13].
Second, sleep has been thought to serve learning and memory consolidation, and performance restoring [8, 14]. This theory proposes that, while memory encoding and retrieval takes place during waking, sleep promotes memory consolidation. During sleep, newly encoded memories are transformed into more stable representations and integrate into the network of pre-existing long-term memories [15, 16]. In addition, an interesting role of sleep regarding performance restoration has been suggested, since studies showed that performance, which was impaired after sleep deprivation, could be enhanced by sleep [17, 18].
Third, sleep has been proposed to serve a glymphatic function [19]. The 'glymphatic system', being termed as such due to its dependence upon glial cells and its performance of peripheral 'lymphatic' functions in the central nervous system, has been described as a biomolecule clearance system that uses convective flow between the cerebrospinal

fluid and interstitial fluid, so that toxic metabolites in the brain are removed, during sleep [19, 20, 21]. Despite the fact that the 'glymphatic system' concept is relatively novel, studies show that failure of glymphatic function is associated with pathology in neurodegenerative disorders, traumatic brain injury as well as stroke occurrence [19, 20, 21].
Fourth and last theory poses that sleep serves a neuronal plasticity function [8, 22, 23]. In this theory, it is proposed that plastic processes which occur during wakefulness lead to a net increase in synaptic strength in many brain circuits, and sleep serves as a mediator to downscale synaptic strength to a baseline level, forming the so-called synaptic homeostasis hypothesis [22, 24].
All aforementioned theories of sleep function have been studied extensively by multiple research groups leading to controversial outcomes, since pros and cons on each theory have been expressed. We spend one third of our lives asleep, and despite the poetically romantic view of Shakespeare on sleep, that we only sleep perchance to dream, although not fully comprehended why, we undeniably need sleep.

1.1.2 Measuring Sleep: the electroencephalogram (EEG)

In the beginnings of the previous century, Hans Berger was the first to publish a series of scientific articles on the remarkable electric effect which he detected in human subjects by recording from electrodes applied to the head [25, 26]. He noted a rhythmic oscillation of potential with a frequency around 10/second, appearing when the subject lies quietly with eyes closed and disappearing if the attention was fully occupied. Berger recorded it by pad electrodes on the scalp or by needles that reached the periosteum of the skull, which he named electroencephalogram (EEG). Since this oscillation was the first one being distinguished from the EEG, it was named the alpha rhythm. Berger concluded that the potential changes were produced by the cerebral cortex and that they represent a fundamental activity of the brain. Although different from humans, potential changes were also found in the exposed cortex of animals [27]. Around the same time, the EEG was used to investigate sleep recordings in humans and animals, mainly in dogs and cats [28, 29, 30].
The EEG signal is the commonly used signal for sleep stages classification. Together with the electromyogram (EMG) for muscle activity and the electrooculogram (EOG) for eye movements recordings, they are excellent biomarkers of sleep/wake states [31]. The EEG and EMG are the recordings of the electrical field activity of large groups of cortical neurons and muscle cells, respectively [32]. The first standard method of sleep recording and manually sleep staging in humans is based on the criteria proposed by Rechtschaffen and Kales [33]. According to them, sleep consists of six different vigilance stages, namely the wakefulness, the non-rapid eye movement (NREM) sleep stage 1 (S1) which is characterized by active muscle tone and slow eyes rolling movements, the NREM sleep stage 2 (S2) in which sleep spindles and K-complexes appear, NREM sleep stage 3 (S3) and NREM sleep stage 4 (S4) in which the responsiveness to external stimuli is really decreased and rapid eye movement sleep (REM) characterized by rapid eye movements and skeletal muscles atonia. During wakefulness, the EEG shows low-voltage fast activity and high muscle tone. As NREM sleep becomes deeper, there is slowing of EEG frequencies and increasing synchronization of cortical neuronal activity, while at its deepest point the neuronal synchrony appears as large slow wave activity on

the EEG [34]. In contrast to NREM sleep which shows high amplitude low-frequency EEG activity, reduced muscle tone and slow rolling eye movements, REM sleep is characterized by low-voltage fast activity, however the muscle tone is entirely diminished (REM muscle atonia), there is cortical desynchrony and the typical rapid eye movements can be distinguished, being associated with dream states [32, 34]. The newer method of sleep staging, developed by the American Academy of Sleep Medicine (AASM), combines sleep stages S3 and S4 of into a single stage, known as slow-wave sleep (SWS) or deep sleep [35]. In humans, healthy night time sleep consists of four to five 90-min cycles of NREM and REM sleep per night with NREM sleep being more prevalent in the beginning and REM sleep more prevalent toward the end of the night. In mammals and other animals, sleep stages may differ, since less elaborate characteristics compared to humans are evident. In rodents, mainly three stages are scored, namely waking, NREM sleep and REM sleep. The parameters extracted from the EEG signal can be used for analysis and classification of the stages of sleep. Specifically, power spectral features, based on Fast Fourier transform (FFT) of the EEG signal, have been used for classification and analysis of sleep stages [36]. Figure 1.1 shows an example of Waking, NREM sleep and REM sleep EEG and EMG recording signals from a mouse after electrode implantation 1.1.
In rodents, in contrast to human polysomnography recordings where EOG is commonly used, mostly EEG and EMG signals are used. Waking EEG, as shown in Figure 1.1, is characterized by the presence of activity between 6-9 Hz, which is thought to arise by a physical spread of theta activity from the hippocampus [37, 38, 39]. Hippocampal theta activity has been associated with voluntary activity, arousal, attention, the representation of spatial position as well as learning. Analogous EEG frequencies are found to characterize REM sleep, also being associated with hippocampal functioning [40, 41]. During NREM sleep, neurons in the cortex and thalamus are bistable, oscillating every second, formulating the distinctive feature of the EEG which is the near-synchronous occurrence of slow waves in the cortical areas prevailing between 0.5-4.0 Hz [42, 43]. Underlying the sleep slow waves, a fundamental cellular phenomenon exists, namely the slow oscillation. The slow oscillation, being the result of disfacilitation (i.e. a lack of synaptic input) consists of an up state, which is characterized by sustained neuronal depolarization and irregular firing, and it is followed by a hyperpolarized down state, during which the cortical cell ceases firing [44, 45, 46, 47]. Due to the fact that the slow oscillations occur more or less synchronously in many neurons, their summed activity is the one recorded from the scalp as slow waves, with the down state of thalamocortical neurons corresponding to the negative phase of the slow waves recorded from the scalp.

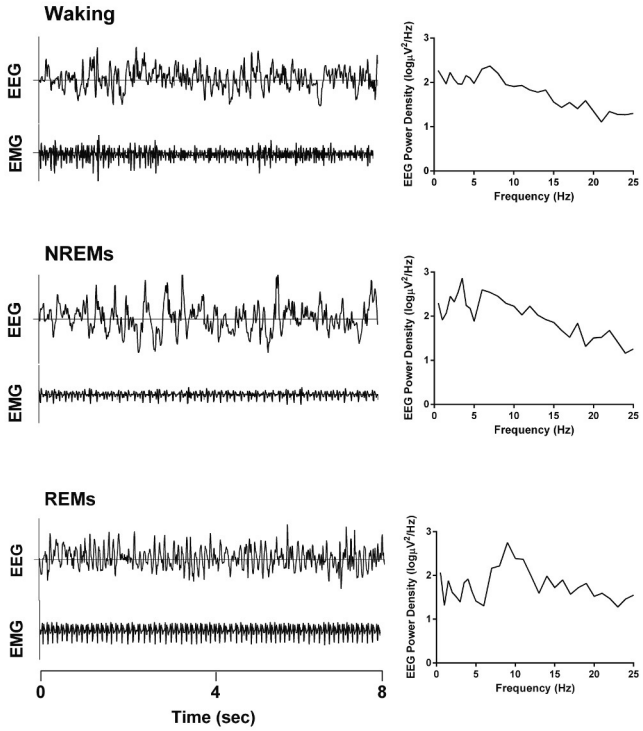

Figure 1.1: An example of raw data of Waking, NREM and REM sleep electroencephalogram (EEG) and electromyogram (EMG) recording signals from a young mouse after electrode implantation.

1.1.3 Sleep and Brain areas

Despite the exponential growth rate of scientific progress, sleep remains one of the most puzzling, yet ubiquitous behaviors across the animal kingdom. One hundred years ago, eminent scientists doubted even the existence of specific neural pathways for wakefulness and sleep regulation. Subsequent research has, however, altered dramatically this picture, and today the neural mechanisms underlying the sleep-wake states have been delineated and are portrayed in the next paragraphs.

Sleep and wakefulness are controlled by a balance between sleep-promoting and wake-promoting nuclei [48]. The ascending arousal system, originating in the brainstem, can arouse the cerebral cortex to promote wakefulness either directly or indirectly via two major branches (Figure 1.2, left panel: A, wake-promoting pathways) 1.2. One branch primarily originates from the pedunculopontine and laterodorsal tegmental nuclei (PPT and LDT) that produce acetylcholine [49], ascending to and activating the relay neurons and the reticular nucleus of the thalamus. Neurons in the lateral hypothalamic area and the basal forebrain are activated by the second branch of the ascending arousal system [50, 51, 52]. Monoaminergic neurons in the upper brainstem and caudal hypothalamus,

including the noradrenergic locus coeruleus (LC), serotoninergic dorsal (DR) and median raphe nuclei, dopaminergic ventral periaqueductal grey matter and histaminergic tuberomammillary neurons, mark the beginning of this pathway.
Markedly, during sleep, the main components of the ascending arousal system become inhibited by projections originating from the ventrolateral preoptic nucleus (VLPO) (Figure 1.2, left panel: B, sleep-promoting pathways) 1.2 [53, 54, 55, 56]. The VLPO can, also, be inhibited by the afferents coming from the monoaminergic arousal systems (Figure 1.2, left panel: C) [57]. A mutual inhibition hence exists, creating this way a flip-flop switch (Figure 1.2, right panel) 1.2. Due to the sharp wake-sleep transitions it allows, the flip-flop switch is rather unstable. Therefore, specific neurons in the lateral hypothalamus, releasing orexin (also called hypocretin) serve to stabilize this switch, via their strong excitatory actions on wake-promoting neurons without disrupting sleep. Finally, REM sleep is generated by neurons around the LC region and sublaterodorsal region in the upper pons. The latter area projects to the basal forebrain and cortex, leading to dream states, as well as to medullary and spinal cord regions in order to inhibit movements during REM sleep [58].

1.1.4 Sleep homeostasis

The concept of a *milieu intérieur*, i.e. internal environment, in physiological processes was first developed in the 19th century, a notion that was later formulated as *homeostasis* [60, 61]. Elaborating, homeostasis, generally, refers to any self-regulated biological process by which organisms tend to maintain internal stability, while adjusting to conditions optimal to survival. The homeostasis term has also been applied to sleep, denoting a basic principle of sleep regulation. Particularly, sleep homeostasis has been described as an internal timer or counter which generates a sleep drive (i.e. pressure to sleep) as a function of the amount of time elapsed since the last adequate sleep episode [62]. In other words, prolonged periods of wake are followed by long periods of deep NREM sleep. This homeostatic response is mediated by NREM sleep-promoting substances, including adenosine, prostaglandin D2, and cytokines such as interleukin-1 and tumor necrosis factor-a [63]. Recent studies have focused on the neuromodulator adenosine, since it is the best understood of these somnogens. A link has been proposed between adenosine, energy metabolism, neuronal activity and sleep [64, 65, 66]. More specifically, adenosine promotes sleep, through A1 and A2a receptor agonists, by directly inhibiting wake-promoting neurons and by activating sleep-promoting neurons. As a result, stimulants such as caffeine and theophylline, serving as antagonists at both A1 and A2A adenosine receptors, can counteract the sleep-inducing effects of adenosine via neurons in the nucleus accumbens possibly projecting to wake-promoting brain regions [67, 68].

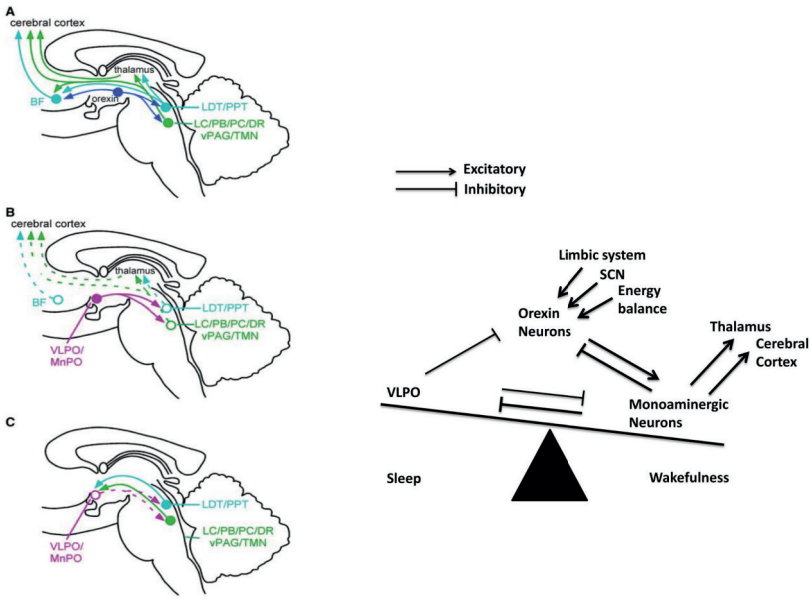

Figure 1.2: *Left panels,* Brain circuits in sleep and wakefulness (*details in the text*). **A.** Wake-promoting projections: Cholinergic neurons (light blue) comprise the major input to the thalamus, while monoaminergic and glutamatergic neurons (green) provide direct innervation of the hypothalamus, BF and cerebral cortex. The orexin neurons (dark blue) reinforce activity in these pathways as well as excite the cerebral cortex and BF. **B.** Sleep-promoting pathways: The sleep-promoting pathways from the VLPO and MnPO (magenta) inhibit the components of the ascending arousal pathways. **C:** The ascending arousal systems are also capable of inhibiting the VLPO. *DR, dorsal raphe nucleus (serotonin); LC, locus coeruleus (norepinephrine); LDT, laterodorsal tegmental nucleus (acetylcholine); PB, parabrachial nucleus (glutamate); PC, precoeruleus area (glutamate); PPT, pedunculopontine tegmental nucleus (acetylcholine); TMN, tuberomammillary nucleus (histamine); vPAG, ventral periaqueductal gray (dopamine).* (*Adapted from Saper et al., 2010* [48])
Right panel, The flip-flop switch during the state of wakefulness: Orexin neurons send excitatory input to monoaminergic neurons, which send inhibitory feedback projection to orexin neurons. This system maintains the activity of monoaminergic neurons. Monoaminergic neurons send inhibitory projection to the VLPO sleep center and send excitatory projections to the thalamus and cerebral cortex. Orexin neurons receive input from the limbic system and also have reciprocal links with the hypothalamic arcuate nucleus that regulates feeding. Additionally, orexin neurons have important role as a link between the energy homeostasis and vigilance states. *VLPO, ventrolateral preoptic nucleus; SCN, suprachiasmatic nucleus.* (*Adapted from Natsuko Tsujino & Takeshi Sakurai, 2009* [59])

Due to the rotation of the earth every 24 hours, living organisms evolved to adopt a *circadian rhythm* which is compatible with the light-dark cycle for their protection, feeding, mating and survival [69]. In humans, the intrinsic period of the circadian rhythm

is slightly longer than 24 h, whereas in mice it is about 23.5 h [70] and, hence, the circadian system comprises a mechanism which synchronizes the internal state of the organism with the predictable environmental changes. Nearly all peripheral tissues in the body have a circadian rhythm which is regulated and synchronized by a central circadian pacemaker, that in mammals is located in the suprachiasmatic nucleus (SCN) of the hypothalamus [71]. The master clock is entrained daily to 24 h by environmental cues, such as light as well as the feeding and activity patterns of the organism [72]. More specifically, the 24-h rhythms are generated by individual SCN neurons and are controlled by a molecular feedback loop [73]. It is important that the activity of these single-cell oscillators is coupled, in order to achieve adequate amplitude to either induce or suppress behavioral characteristics for specific phases of the cycle [69]. Among many physiological processes occurring in the body, the circadian clock controls also the sleep/wake cycle [71]. Unlike the aforementioned homeostatic control of sleep, circadian-controlled processes are driven by an autonomously rhythmic system. It has been proposed that the SCN is possibly unique in the entire mammalian brain since its neurons constitute a coupled, intercellular network of self-sustained, circadian oscillations of both neuronal activity and gene expression, even in the absence of external stimuli [74, 75]. Circadian rhythm outputs can be measured in many ways, for example the rest-activity rhythm can be measured using actigraphy over several days [76].

The timing, depth and duration of sleep are considered to be regulated by the interaction of these two processes, the sleep homeostatic and circadian process (*Process S and C*, respectively) as proposed by the so-called two-process model of Borbely [77] (Figure 1.3) 1.3. Process S, representing sleep debt, increases during wakefulness, declines during sleep, and oscillates with a periodicity normally entrained to day and night by the circadian pacemaker. When S is at the lower boundary, it triggers awakening whereas near the upper boundary it triggers sleep.

In mammals, the homeostatic sleep process is thought to be reflected in the NREM sleep electroencephalographic (EEG) slow-wave activity (SWA), which is the EEG power density between 0.5-4.0 Hz. EEG SWA increases in the course of waking, having its maximum level at the beginning of the sleep period, and declines during sleep showing its lowest values just before awakening [78, 79]. EEG SWA consists of two rhythms that have different brain origin and dissimilar neuronal mechanisms [80, 81]. The first one is the slow neocortical rhythm (<1Hz) which is generated within the cortex [80]. Through high-density EEG recordings, it has been shown that each cycle of the slow oscillation reflects a traveling wave which originates in prefrontal-orbitofrontal sites and propagates towards more posterior cortical areas [42]. The important role of the cortical slow oscillation is to synchronize the activity of cortical and thalamic neurons that generate spindle and delta waves throughout NREM sleep [82]. The second rhythm refers to a faster oscillation in the slow frequencies (1-4 Hz) generated in the thalamus, specifically by the interplay of two intrinsic currents of thalamocortical neurons [47]. SWA being the combination of these two rhythms is widely considered to be a measure of sleep need and/or intensity. Therefore, in the event of prolonged wakefulness, SWA increases in the subsequent sleep period in both humans and animals [78, 79]. The dynamics of sleep regulation have been extensively studied in humans and animals with the use of mathematical modeling of the observed homeostatic sleep response, visible in the EEG SWA, before and after sleep deprivation [83, 84, 85, 86]. In the next section, the effects and

applications of sleep deprivation are discussed.

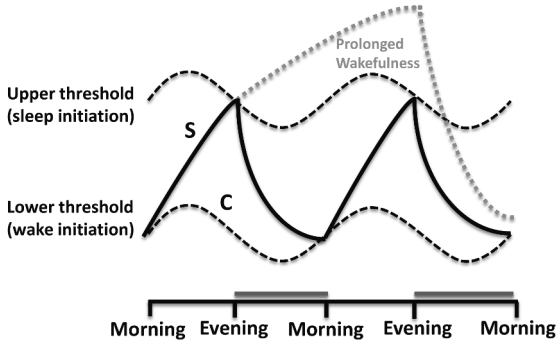

Figure 1.3: The sleep-wake regulation: the interaction between sleep homeostatic (Process S) and circadian process (Process C). Prolonged wakefulness increases sleep pressure, leading to increased total sleep time and slow-wave-activity in NREM sleep (SWA) in recovery sleep. (*Adapted from Borbely et al., 2016* [78].)

1.1.5 Sleep deprivation

In humans, the physiological sleep drive can be overruled by voluntary sleep restriction, or it can be disrupted owing to environmental factors and/or sleep disorders, for example obstructive sleep apnea, insomnia, restless leg syndrome, narcolepsy [87]. In general, sleep disruption or deprivation is detrimental for health and well-being [88]. Numerous findings in the last decades support this notion. Notably, even one night of sleep deprivation, increases cortisol levels and potentiates the HPA axis [89]. Night shift work, a common work schedule in a variety of professions, is associated with short sleep of less than 5 h [90, 91]. Sleep disruption or deprivation has been shown to impact metabolism and the immune system [87, 92, 93, 94, 95], with short sleep being associated with an increased prevalence of type 2 diabetes, obesity, and cardiovascular disease [96, 97, 98, 99]. Furthermore, cognitive performance is compromised following acute and chronic total sleep deprivation but also chronic sleep restriction, showing that sleep debt has a neurobiological cost which accumulates over time [100]. In animal studies, it has been demonstrated that sleep fragmentation impairs memory consolidation [101] and is a risk for Alzheimer's disease [102, 103]. In the most extreme case, continuous sleep deprivation can even kill rodents and flies within a period of days to weeks [104]. In order to test the sleep homeostatic process and investigate sleep characteristics under elevated sleep pressure conditions, sleep deprivation has been experimentally used in the laboratory, as some of the earlier studies, reported in this section, showed. The increased sleep pressure, as an effect of sleep deprivation, leads to alterations in the sleep-wake regulation which consist of increased total sleep time, accompanied by EEG changes

(Figure 1.3) 1.3. The sleep rebound, i.e. the sleep recovery following sleep loss, is characterized by increased sleep duration and intensity. A possible reduction in the recovery sleep, which is typically quantified as the percentage of sleep lost that is recovered over a defined time period, could underlie an impaired homeostat, unable to provide sufficient sleep or a highly efficient homeostat that is capable of restorative effects with very little sleep [105]. As aforementioned, SWA in NREM sleep is a well established electrophysiological marker of sleep homeostasis in mammals and a quantitative measure of the amplitude and incidence (number per minute of NREM sleep) of sleep slow waves [106] and it is found to be increased following sleep deprivation.

1.2 Aging: theories, sleep and the brain

1.2.1 Aging theories

Ambrosia and *Nectar*, nutriments once thought to grant immortality, are not to be found on Earth for human beings. Antithetically, aging and mortality signify an endpoint that almost all organisms on the planet experience. Potential absence of aging composes the ideal concept of biological immortality. Although this is utopian for human beings, it is actually incarnated in simple organisms such as the *Hydra* genus, which does not appear to die of old age, or to age at all, having a regenerative ability [107]. The question therefore arises, is it the complexity of higher-order creatures that enables them to senesce?
Philosophers, from the beginning of cultures, have dealt with the deeper idea and understanding of aging. A writer and philosopher of modern times said: *"In the temporary living beings, two streams struggle, first the upslope towards synthesis, life and immortality, and second, the downslope towards decomposition, matter and death" (Nikos Kazantzakis) [108].* From a scientific point of view, attempts have been made in order to elucidate the nature of aging and more importantly, whether aging can be reversed. Generally, aging can be defined as the condition where all physiological mechanisms decline, rendering an organism closer to its "death".
Aging comprises a markedly complicated and multifactorial process, thus numerous theories have been proposed over the years [109]. These can be classified into several groups of theories that may overlap at various levels of organization, being sometimes complementary with each other.
One group of theories, known as the "error theories", generally identifies several environmental insults to organisms, inducing progressive damage at various levels, e.g. mitochondrial DNA damage, oxygen radicals accumulation, cross-linking [109, 110]. The "general error theory" suggests a decline in the fidelity of gene expression with aging, leading to increased fraction of abnormal proteins [111, 112]. This erroneous synthesis can in turn generate a positive feedback of errors ultimately leading to a lethal 'error catastrophe'; however, despite the presence of error, there may be some stability of translation achieved [111, 112, 113].
Another theory which can also be categorized in the "error theories" group and has received a lot of attention, is the "free radical theory". First proposed in 1950's, it suggests that, the release of active free radicals eventually results in cumulative damage and senescence [114]. These free radicals are naturally produced by the organisms during energy production, in which mitochondrial respiration generates reactive oxygen species (ROS)

by leaking intermediates from the electron transport chain, rendering the internal environment of all living organisms into a container of free ROS [115]. Interestingly, elevated levels of oxidant-damaged DNA as well as protein have been found in aged organisms [116, 117].
The main rival theory to the "error theories" poses that aging is genetically programmed in a similar way as morphogenesis. According to these so-called "programmed theories", aging depends on biological "clocks" that regulate the time of the life span based on genes that sequentially switch on and off signals to the nervous, endocrine, and immune systems being in turn responsible for maintenance of homeostasis and for activation of defense responses [109, 110].
Furthermore, "evolutionary theories" have been formulated, in which it is supported that the deterioration observed in the course of aging results from a decline in the force of natural selection [110, 118]. In contrast to the previously discussed theories, in the evolutionary theories, aging is not programmed but an accumulation of somatic damage occurs due to limited investments in maintenance and repair beyond the age of reproduction [119].
In order to understand the insights of the aging process especially for organisms such as mammals, being extremely complex, the overall interactions among intrinsic (genetic), extrinsic (environmental), and stochastic (random damage to vital molecules) causes should be studied in a more holistic approach [110].

1.2.2 The Aging Brain and Sleep

In the course of aging, brain alterations are evident in multiple levels, from molecules to morphology and brain size. Total brain volume is shown to decline with age [120], at a rate of around 5% per decade after the age of 40 [121]. Advanced age has also been associated with widespread thinning of the cerebral cortex [122]. With respect to specifically the neocortex and hippocampus, the last decades, the idea that age-related decline in neuron number through neuron death is involved in normal aging, has become less popular [123]. The prefrontal cortex of the brain has been demonstrated to be mostly affected in healthy aging, however, in both rodents and humans, changes have been reported in dendritic arbor, spines, and synapse morphology that could impact the function of hippocampal circuits, rendering also the hippocampus another greatly affected area [123, 124, 125, 126, 127]. With regards to the synapse, loss of synaptic function and number is probably also a contributing factor in age-related cognitive decline [128, 129].
In addition, disruption of particularly myelinated fibers that connect neurons in different cortical regions occurs in the aging brain, suggesting a systems-level breakdown of integrated function that correlates with poor cognitive performance [130]. Aging has also been shown to affect white matter density, with great reductions found in the prefrontal cortex and anterior corpus callosum [131, 132].
Neurotrophic factors likely play an important role in the aging brain, since increased concentrations of them can restore cognitive functions in aged animals [133]. For example, the expression of brain-derived neurotrophic factor in the hippocampus has been found to decrease with age, an effect that may contribute to age-related cognitive impairments [134]. In addition to neurotrophic factors, neurotransmitters playing naturally an impor-

tant role in the brain, show alterations in the course of aging, that could ultimately lead to neurodegenerative diseases. Recent research has identified serotonin as a prominent signal, acting in concert with brain-derived neurotrophic factor, in order to regulate aspects of neural plasticity in several brain regions. It has been demonstrated that both could become compromised in aging and age-related neurodegenerative disorders [135]. Furthermore, human postmortem and animal experimental results suggest an age-associated disturbance of the cerebral dopaminergic neuronal system [136, 137, 138, 139, 140]. Particularly, dopaminergic pathways between the frontal cortex and the striatum, levels of dopamine may decline as a function of age, and/or synapses/receptors or binding to receptors may be reduced [141]. In general, cholinergic and monoaminergic systems projecting from the basal forebrain and brainstem have been demonstrated to be functionally impaired in the course of aging, with the metabolites of achetylcholine, dopamine and noradrenaline being generally depleted in the cerebral cortex of aged rats and monkeys [142, 143].

In the aged brain incomplete or impaired functions can be detected. Stemming from interrupted pathways and brain atrophy, sleep in aging is associated with a disrupted physiology [144]. Particularly, sleep quality is considered to be progressively decreased in the course of aging in humans, owing to reduced capacity to initiate and/or maintain sleep. The elderly sleep less, reporting more often sleep complaints, and their sleep is accompanied by frequent awakenings and superficial stage 1 sleep [145, 146, 147]. The sleep EEG is characterized by lower slow-wave activity in NREM sleep, reflecting a less deep sleep, as well as reduced sigma activity (around 13 Hz) during NREM sleep, beginning at middle age [148, 149, 150].

The brain mechanisms contributing to age-related sleep alterations are not fully understood to date. A preferential impairment of the prefrontal cortex evident from neuropsychology and brain imaging techniques has been noted with age, that may intervene with the sleep process [144]. The medial prefrontal cortex expresses gray matter reductions in elderly subjects, and comprises an area where NREM sleep slow waves show a marked preponderance in origin and density over EEG derivations [144, 151]. Compared to young adults, older subjects show lower slow-wave amplitude and density, especially in prefrontal/frontal brain areas [42]. In addition, serotonin, being implicated in a variety of neural functions including sleep regulation [152], may contribute to age-related sleep alterations, since diminished populations of serotonin receptors with age have been demonstrated in rat and human brains [153]. Interestingly, changes in the suprachiasmatic nucleus in the hypothalamus, as well as other parts of the circadian timing system are considered to underlie sleep disturbances in elderly people [154, 155]. In particular, the aging SCN is characterized by decreased neuronal activity [156, 157], showing an attenuated circadian amplitude, due to changes in neuronal phase distribution [158]. Finally, vasopressin, one of the main peptides in the SCN, declines in amount and synthesis in aged humans, leading eventually to disturbed sleep-wake rhythms [159].

Concluding, aging is a rather slow process that potentially impacts all the organs including the brain. Along with many physiological processes, sleep is consequently largely affected, rendering the elderly vastly vulnerable, as sleep remains a vital physiological process.

1.3 Environmental factors, health and aging

The so-wanted elixir of youth has not been discovered yet. However, many factors may intervene in the aging process, leading to detrimental or beneficial effects, which in turn could accelerate or decelerate parts or the whole aging procedure. Aging is a physiological process that occurs asynchronously in different areas of the brain and the rate of that process is dependent on the lifestyle of the individuals. In the following paragraphs, some of the environmental factors that humans are daily exposed to are discussed.
First, diet constitutes a significant factor, directly affecting general body and brain health. In addition to health, longevity per se is believed to be promoted through diet [160, 161, 162]. Dietary interventions, such as caloric restriction and intermittent fasting can improve health during aging, reduce the risk of developing diabetes, cancer, and cardiovascular diseases and even delay brain atrophy evident in aging [163, 164, 165, 166]. A disorder commonly viewed across all ages is obesity. Obesity can be accompanied by damage to several target organs, as for example the heart, skeletal muscle, pancreas, liver, and kidney [167]. Additionally, in human obesity, structural brain changes have been identified in several brain areas [168], rendering obesity a risk factor for neurodegenerative disorders, such as Alzheimer disease [169]. Therefore, in congruence with the notion that the choice of our food defines us, diet reflects life quality.
Second, since the 1900's artificial light was introduced as another important factor that influences our daily lives. Although artificial light has ameliorated human life in a multitude of sectors, augmented exposure, particularly during times that darkness should prevail, has been associated with drawbacks in health [170]. General disruption of normal circadian time can alter the overall physiological state. Hormone balance, immune, endocrine, as well as several metabolic system disturbances may be provoked owing to light exposure at night. Shift-work, a widely-accepted type of work in modern society, can induce detrimental effects in carbohydrate and lipid metabolism, insulin resistance, hypertension, coronary heart disease, and myocardial infarction [171], being linked also with increased risk for breast cancer [172, 173, 174, 175]. Hence, artificial light exposure, specifically at night, impacts physiological processes, that may affect consequently body and brain health.
Third, physical activity constitutes one factor, that exhibits large attention regarding general health. The roots of a mind-body connection are traced back to ancient civilizations. Plato said, "*In order for man to succeed in life, God provided him with two means, musical education and physical activity. Not separately, one for the soul and the other for the body, but for the two together. With these two means, man can attain perfection" [176].* Physical activity, such as aerobic exercise, are implicated in cognition, memory and mental health improvement, as well as in promoting structural and functional plasticity in the brain [177, 178, 179, 180]. The elderly can essentially benefit by exercise, since cognitive functioning can be particularly enhanced [181, 182]. Additionally, it has been suggested that the structural and functional benefits of exercise can even mimic those of antidepressants [183, 184]. Thus, a prominent role of physical activity in general body and brain health augmentation has been developed.
Finally, substances such as caffeine, widely used in a daily basis, correspond to environmental factors that may directly affect health. Mainly consumed in order to promote alertness and increase wakefulness, caffeine leads to very important secondary effects

on many classes of neurotransmitters, after blocking adenosine receptors, influencing in turn a large number of different physiological functions [185]. Lifelong caffeine intake has been associated with prevention of cognitive decline, and reduced risk of developing stroke, Parkinson's disease and Alzheimer's disease [186, 187, 188, 189, 190]. Caffeine, therefore, being regularly consumed, might serve body and brain health enhancement qualities.
Considering the amount of time that every individual is directly or indirectly affiliated with these environmental factors, delving into their features and actions might prove beneficial. Therefore, environmental factors, such as the ones addressed, to which daily exposure has been noted, may assist in promoting general health and healthy body and brain aging.

1.4 Aim of the current thesis

The current thesis aims to shed light on long-term effects of environmental factors on sleep and the sleep EEG, in the mouse model. *Diet, physical activity, light levels and caffeine intake*, comprise considerable environmental factors in daily life. Investigating the beneficial or adverse effects following long-term exposure to them not only during early adulthood but also in aging provides significant insights that could accelerate, hinder or ameliorate parts of the aging process allowing for a healthier and longer life span.
This thesis is partitioned into two parts, namely the young and the aging part. In particular, **Chapters 2, 3, 4** concern studies conducted in young mice, whereas **Chapters 5, 6, 7, 8, 9** studies conducted in aged mice.
Obesity is associated with sleep disturbances that may interfere with general life quality. Therefore, in **Chapter 2** sleep regulation is studied following diet-induced obesity in young mice. In order for obesity to be developed in mice, so that it simulates the human condition, mice are fed for 12 weeks exclusively with high-caloric diet. The effect of high-caloric diet on sleep homeostasis is tested with the use of parameter estimation analysis and simulations of the sleep homeostatic process S.
In **Chapter 3** the influence of caffeine consumed either acutely or chronically (for two weeks continuously) on sleep, the sleep EEG and behavior is studied in young mice. Although the effects of acute caffeine intake are largely known, inducing almost instantly alertness, caffeine is usually consumed over a longer period of time, and this latter condition has not received much attention from researchers. Thus, in this study caffeine is diluted in the water in order for the mice to have continuous access to it for at least two weeks.
Dim-light-at-night interrupts sleep and circadian rhythms. Therefore, **Chapter 4** focuses on the impact of acute and long-term exposure to dim-light-at-night on sleep, the sleep EEG and behavior in young mice. Particularly, mice are exposed to one night, one week, one month and three months to a light:dim-light-at-night 12:12 h schedule.
Chapter 5 comprises the first of the series of chapters concerning sleep studies in aging. Therefore, in this chapter, sleep and the sleep EEG as well as the morphology of sleep slow-waves is studied in the course of aging in naturally aged mice. The basic detailed effects of aging are hence established, so that the impact of environmental factors in the

chapters that follow can be investigated.
In **Chapter 6** the effect of long-term physical activity on sleep, sleep EEG, and behavior is studied in young and older mice. Exercise, generally, has been proposed to be an alternative to pharmacological treatment for sleep disorders. Hence, in this study, a running-wheel is available in the cages of the mice for one to three months prior to sleep recordings. The sleep recordings are performed with the absence of the running wheel, studying this way the sustained effect of exercise in the course of aging.
In **Chapter 7** the influence of diet-induced obesity on sleep is studied in aged mice. 18 and 24 months old mice are fed exclusively with high-caloric diet for continuous three months prior and during the sleep recordings. This study, therefore, investigates potential obesity-related sleep disturbances in older mice which are eventually compared with the effects in young mice studied in **Chapter 2**.
Chapter 8 investigates the effects of long-term dim-light-at-night exposure on behavior, sleep and the sleep EEG in aged mice, emphasizing the differences from the young mice studied in **Chapter 4**. Thus, 18 and 24 months old mice are exposed for continuous three months to a light:dim-light-at-night schedule.
In **Chapter 9** sleep and the sleep EEG is studied in a genetic mouse model. The main characteristic of this mouse model is the premature aging due to a gene elimination which has a function in DNA repair, rendering the life span of mice to maximum 18 weeks. The data emerging from this mouse model are subsequently compared to data obtained from naturally aged mice, in order to investigate whether this model can produce similar results and eventually being able to replace the natural aging procedure in aging research.
In **Chapter 10** data from all the studies performed are discussed, addressing the main issue of the present thesis, can health in the course of aging be enhanced?

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