Homeostatic regulation of wakefulness

-A model and hypotheis driven approach

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Forord

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Du har oppmuntret meg hele veien, lært meg utrolig mye og vært veldig tålmodig med meg. Jeg kunne ikke bedt om en bedre veileder.

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Sammendrag

Søvnhomeostase-modeller forsøker å forklare hvordan og hvorfor hjernen øker lengden på søvn etter en lengre periode i våkenhet. Flere av disse modellene foreslår at søvn har en restituerende funksjon på hjernemetabolismen vår, både ved å fjerne metabolske avfallstoffer og ved å gjenopprette oppbrukte energilagre. Studier i de siste årene har vist at lengde og intensitet på søvn kan øke som en funksjon av aktiv våkenhet. I tillegg er det blitt påvist elektroencephalografiske markører av søvnhomeostase under rolig våkenhet. Et mindretall av nyere studier foreslår også at metabolsk restituerende funksjoner av søvn kan være effektive i rolig våkenhet. Disse funnene åpner opp for en interessant mulighet; at den homeostatiske reguleringen av søvn kompletteres av en homeostatisk regulert stille våkenhet. Denne muligheten ble utforsket ved å utvikle og å teste en homeostatisk modell av våkenhet. Basert på denne modellen ble det utført en hypotesedrevet analyse for å undersøke om homeostatisk regulert rolig våkenhet kunne predikere lengre våkenhet hos rotter. Basert på analyse av to rotter ble det mistenkt at høyfrekvent gamma aktivitet kunne identifisere homeostatisk regulert rolig våkenhet, men denne hypotesen ga blandede resultater i en større populasjon med rotter.

Abstract

Sleep homeostasis models attempt to explain why and how the brain responds by increasing duration and intensity of sleep as a function of intensity and duration of prior wake. Several lines of research within this paradigm suggest a restorative metabolic function of sleep: restoring resources depleted during wake or clearing waste products that accumulate during wake. Increasingly, homeostatic sleep pressure is being viewed as accumulating at a higher rate during active waking, as compared to quiet waking. In addition, electroencephalographic markers of sleep homeostasis have also been shown to occur during quiet wakefulness. Finally, a handful of studies also suggests that some of the same restorative metabolic functions as in sleep may be effective during quiet wakefulness. These observations suggest an intriguing possibility that the homeostatic regulation of sleep is complimented by a similar regulation in quiet wakefulness. Here, this possibility is explored by developing and testing a homeostatic model of wakefulness. The model suggests that quiet wakefulness may increase the ability to sustain wakefulness. A hypothesis driven approach identified high intensity-, high frequency gamma as a possible marker of quiet wakefulness homeostatic regulation. This analysis represents a first attempt at identifying the hypothesized homeostatically regulated quiet wakefulness. The results were ambiguous, as the findings did not generalize to a larger sample of rats.

Key words: active wakefulness, quiet wakefulness, sleep homeostasis

Innholdsfortegnelse

By being awake, we perform a multitude of behaviors necessary for survival and reproduction. These behaviors include procreation, learning, rearing of offspring, gathering food, exploring, detecting and escaping from predators and more; all contributing to reproductive success. Reproductive success is the net amount of genetic copies of the individual which is passed on to the next generations. Increasing the amount of genetic copies may be achieved through sexual reproduction, or indirectly through increasing the amount of copies other individuals with the same genes are able to reproduce. Reproductive success also relies on survival. The net effect on reproductive success of a behavior is considered the adaptive value of that behavior. If a behavior has high adaptive value, it is an adaptive behavior. Since many adaptive behaviors rely on being in a state of wakefulness, wakefulness is a highly adaptive state.

As wakefulness is extended over time, it may become less adaptive. People who are kept awake for longer than normal (i.e 16 to 18 hrs) through sleep deprivation show increased levels of sleepiness and negative mood (Franzen et al., 2008), while performance on motor, cognitive and attention-related tasks decrease (Goel et al., 2009; Pilcher & Huffcutt, 1996). In a study by van Dongen and colleagues (2003), groups of adults were either kept awake 24 hours for 3 days or had their sleep restricted to 8, 6 or 4 hours for 14 days. After wake

extension, the subjects displayed impairments on cognitive performance, sleepiness and attention-sustaining tasks which intensified with the amount of wake extension. The effects of these impairments carried over across multiple days, but also varied with the time of day and inter-individual differences.

Impairments in performance due to wakefulness extension may be fatal. As an example, reduction of performance by sleep deprivation increases the risk of human-error related car accidents (Dinges, 1995). Repeated and chronic extended wakefulness may also cause negative health outcomes. Extended wakefulness is correlated with increased concentrations of toxic waste, such as amyloid β (Aβ). Aβ is a protein released in increasing amounts during higher signaling activity. Accumulation of Aβ is associated with formation of aβ-plaques, which is currently understood to contribute to development of Alzheimer's disease (Sadigh-Eteghad et al., 2015). This suggests a causal link between the reported association of wakefulness extension and increased risk of Alzheimer's disease (Palma et al., 2013; Wu et al., 2019). Due to the implications for performance and health, figuring out why adaptive wakefulness is limited, and how capacity of adaptive wakefulness is restored is a crucial objective of research.

A model of how effective adaptive wakefulness is maintained is presented. Reported decreases in health- and performance outcomes of extended wakefulness suggest that the adaptive value of wakefulness is a capacity limited in time and use. In addition, evidence is presented supporting that sleep and quiet wakefulness are involved in restoring this capacity. Based on this model, I predicted that markers of homeostatic regulation in quiet wakefulness are associated with capacity to sustain longer periods of wakefulness in rats. This prediction was investigated with a hypothesis driven approach. Ultimately, an attempt at identifying homeostatically regulated quiet wakefulness based on EEG and EMG markers yielded ambiguous results.

1.1 What is adaptive wakefulness capacity?

The concept of a "wakefulness capacity" was introduced by Kleitman (1964), as the length of wakefulness sustained throughout a 24-hour day-night cycle. He estimated the absolute length of human waking capacity to reach 15-17 hours for normal adults. The word "capacity" implies a limit, and as Kleitman himself noted, wakefulness can be expanded well beyond 17 hours. Recently, Vyazovisky (2015) introduced the concept of "adaptive wakefulness" as "a condition whereby the organism interacts effectively with the environment and is capable of maintaining normal physiological waking functions within strict physiological limits, which altogether increases chances of survival and successful reproduction". (Vyazovskiy, 2015, p. 172)

Considering that the length of wakefulness can be extended over several days for humans (Gulevich et al., 1966; Waters et al., 2018), it seems that the time-limit of the wake capacity is in the extent of adaptive wakefulness. Here, these concepts are combined to define a novel concept, adaptive wakefulness capacity; the limited amount of adaptive wakefulness an animal may sustain throughout a 24-hour cycle (in time or use).

1.2 What are the limits described to date?

Establishing the limits of adaptive wakefulness requires detection of sleep since this represents the alternative state to wakefulness (omitting torpor and hibernation). Sleep is a complex physiological state of reduced responsiveness to the external environment (Cirelli & Tononi, 2008). The occurrence of sleep highlights that it serves an adaptive function. Sleep would likely be eliminated by natural selection if it did not serve an adaptive function (Cirelli & Tononi, 2008). Since the global state of sleep is incompatible with wakefulness; it logically follows that wakefulness is not adaptive at the same time as sleep. Thus, if an animal engages in global sleep, it may be assumed that the length of adaptive wakefulness is limited. Even in

animals who may stay continuously awake, such as sea-living mammals and birds, adaptive wakefulness cannot be maintained continuously. Instead of global sleep, these animals exhibit unihemispheric sleep, where one hemisphere is unresponsive while the other remains responsive (Mascetti, 2016). All animals studied to date have revealed a state of global sleep, sleep-like states (rest states), and unihemispheric sleep when measured objectively. For this reason, sleep is considered a near-universal (Keene & Duboue, 2018) or universal (Cirelli & Tononi, 2008) phenomenon. Consequently, adaptive wakefulness is time-limited for all animals studied.

1.3 Wakefulness versus sleep

States of wakefulness and sleep are objectively identified using electroencephalographic (EEG), electromyographic (EMG) and electrooculographic (EOG) data. For mammals and birds, researchers distinguish between wakefulness, non-rapid eye movement sleep (NREM) and REM sleep. NREM sleep is the most inactive and dominant state of sleep, while REM sleep is a particularly active form of sleep. These states are scored based on standardized criteria. Sleep is identified using similar methods specific for different animals. As an example of the similarities and differences, human and rat scoring are presented.

In humans, wakefulness and sleep may be visually scored by American Acadamy of Sleep Medicine (AASM) guidelines (Silber et al., 2007). Intervals of fixed length (epochs) are scored by the dominant EEG and EMG signals. Wakefulness is defined as epochs where alpha (8-13 Hz) or low amplitude mixed frequency (2-7 HZ) signals dominate. Wakefulness transitions into NREM sleep. NREM sleep is divided into stages N1, N2 and N3. The first stage of sleep that occurs after wakefulness terminates is the transitional stage of N1. N1 is defined as the epoch with the first observed 4-7 Hz activity in the absence of alpha, with a

slowing of frequencies in the ≥1Hz range after wakefulness. N2 is a stage rich in K-complexes and spindles, which are morphologically distinct EEG features. From N2 there is a progressive transition into N3. N3 is a stage referred to as slow wave sleep (SWS), defined as sleep dominated by high amplitude activity in the slower (0.5-2Hz) frequency range. Finally, the N3 and N2 stages may transition into REM sleep. The state of REM is defined as when chin EMG tone falls. It is differentiated from N2 by absence of K-complexes, spindles and presence of rapid eye movements or low amplitude mixed frequency EEG.

Norway's first sleep researcher, Reidun Ursin, developed a standardized method of staging sleep and wakefulness in rats together with her collaborators (Neckelmann et al., 1994; Neckelmann & Ursin, 1993; Ursin & Larsen, 1983). As in human sleep scoring, the method is used to differentiate between wakefulness, three stages of NREM, and REM sleep. Rat wakefulness is characterized by low amplitude-, desynchronized-, fast frequency activity (up to 40 Hz). Wakefulness transitions into NREM, which is scored as three different variants: A transitional type sleep, SWS-1 and SWS-2. SWS1 is characterized by the presence of spindles. It is distinguished from SWS2 by a lower presence of slow wave activity (SWA;0.5-4Hz). High amplitude slow wave activity (SWA) dominates SWS-2. Finally, REM sleep is characterized by similar EEG characteristics as in wakefulness; low amplitude, fast frequency activity, and moderate 6-9 Hz activity. Identifying REM is still possible, due to a prominent silencing of the EMG tone (muscle atonia), except for short bursts of activity typically associated with rapid eye movements.

In a validation study, Neckelman and Ursin (1993) compared the arousal threshold (the amount of external stimuli needed to elicit a response) of each sleep state. To measure the arousal threshold, they exposed rats to auditory stimulation during sleep. Responses were measured by brief responses in EMG and EEG changes corresponding to an arousal (desynchronized activity, sleep-spindle suppression, EMG tone increase). During SWS-2, the

arousal threshold was significantly greater than all the other sleep stages. They also found that epochs of NREM with higher delta power were associated with higher arousal threshold, as has been reported for humans (Rechtschaffen et al., 1966).

The similarities between rat and human EEG and wakefulness and sleep characteristics suggest an evolutionarily conserved regulation (Léger et al., 2018). An evolutionary conserved electrophysiological regulation of wakefulness is not unique to humans and rats, but is found among cats, bats, mice and dogs, among others (Buzsáki et al., 2013). The likely common evolutionary origins may justify using different species to study wakefulness. Classes of other animals, such as reptiles, are different to mammals in sleep regulation. In these animals the state of REM sleep is either not present or not identified (Libourel & Barrillot, 2020).. Thus, studies of mammalian wakefulness and sleep are more likely to compare the same underlying physiology than reptile wakefulness and sleep.

1.4 What is normal wakefulness?

An approximate estimate of adaptive wakefulness capacity is obtained by establishing what normal range of wakefulness-length an animal of a species sustains in an environment equivalent to where the behavior evolved. Since methods that can track wakefulness (and sleep) over longer time are largely incompatible with studies in natural environments, most studies are performed in laboratory conditions. The laboratory settings are typically controlled with important implications for wakefulness regulation. For instance, the animals have access to food and water, there are no predators, and they are habituated to a sensory deprived environment with limited options of motility and often little or no possibility of social interactions. Since the behavior of the animal in laboratory settings is highly artificial, it cannot be inferred that such regulated wakefulness length is adaptive.

For the time being, studies outside of the lab rely on observational data, measurements of activity/inactivity (actigraphy), distance moved (GPS-tracking), or less accurate and spatially limited telemetric identification of sleep and wakefulness. This challenges the validity (sensitivity, specificity and accuracy) of the data (Rattenborg et al., 2017). Furthermore, a lack of control of the environment challenge data to be reproduced (reliability). The difference between laboratory and wild conditions can be massive. For instance, the sloth *Bradypus variegatus* was originally believed to spend about 8 hours a day awake. But when Rattenborg and colleagues (2008) were able to record their brain activity in the wild, they observed more than 6 hours of additional wakefulness, almost doubling the captive estimate.

Despite these limitations, a comparison of the length of wakefulness between and within species to estimate which parameter that could be key to determine adaptive wakefulness capacity may be reasonable.

2.1 How is wakefulness regulated?

There are large variations in the length of wakefulness between species, as revealed by phylogenetic comparisons of sleep-duration (Siegel, 2005). For instance, the African elephant is reported to stay awake for about 18-20 hours, while the big brown bat is only awake for about 4-6 hours each day (Siegel, 2005). In mammals, the duration of wakefulness is associated with the type of food intake. Herbivore diets correlate with the longest time in wakefulness and carnivore diets with the least amount of wakefulness. Omnivores are found to show time in wakefulness in between herbivores and carnivores (Siegel, 2005). Within the herbivores, body size positively correlates with longer duration in wakefulness. For omnivores and carnivores this relationship both non-significant (Siegel, 2005), and significant relationships are reported (Savage & West, 2007). The differences in results may be a result

of exclusion criteria, as Siegel (2005) did not include marine mammals in his estimates(Savage & West, 2007).

Some reports suggest that bigger brain size also shows a positive correlation with wakefulness, explaining most variation in duration by body size (Lesku et al., 2006; Savage & West, 2007). While this view is debated (Siegel, 2009), the relationship between brain size and longer time in wakefulness in non-primate mammals is by some researchers explained by a) the number of cortical neurons, and b) density per surface area (Herculano-Houzel, 2015). Increased density per surface area is associated with decreased wakefulness length, while the opposite relationship exists for number of cortical neurons. Primates, including humans, may be considered outliers since they sleep longer than mammals with bigger brains (Herculano-Houzel, 2015).

Ontogenetical differences may also influence the adaptive wakefulness length. As an example, newborn human babies are only awake for about 8 hours (Parmelee Jr et al., 1964), while adult humans are typically awake for 16-18 hours. In addition, for adults, the wakefulness is consolidated mainly in one long period (monophasic), while for newborns it consists of many (polyphasic), sustaining about 2-3 hours without sleep at the longest (Parmelee Jr et al., 1964).

Thus, when comparing regulation of adaptive wakefulness length, between-species and ontogenetic differences must be considered. Despite these associations, most of the variation between and within species remains unaccounted for by phylogenetic comparisons. Since mechanisms of wakefulness and sleep regulation are highly conserved among species of different phyla (Eban-Rothschild et al., 2018) adaptive wakefulness capacity may best be understood by investigating how wakefulness is regulated.

2.2 Global wakefulness and local arousal

Wakefulness is a dynamic state regulated through global and local variations in arousal. Arousal refers to the non-specific activation during sleep or wakefulness, and is closely related to the degree of vigilance and alertness of the animal (Oken et al., 2006). Wake-promoting neurons originate from the brain stem, through the midbrain, and split into two central wake-regulating pathways. The ventral pathway innervates the hypothalamus, basal forebrain and the cortex, acting in concert to excite the cortex. A dorsal pathway innervates the thalamus, facilitating transmission of sensory information to the cortex. Wakefulness is suggested to occur when both the ventral and dorsal pathway are activated (Eban-Rothschild et al., 2018).

These populations house monoaminergic and cholinergic neurons. Monoaminergic neurons mainly signal through the neurotransmitters dopamine, norepinephrine, serotonin and histamine, while cholinergic neurons rely mainly on acetylcholine for signalling (Eban-Rothschild et al., 2018). The hypocretin neuropeptides are also considered essential signalling molecules, acting to modulate the activity of all other wake promoting populations. Glutamate signialling may be involved in both activating and inhibitory regulation of wakefulness (Eban-Rothschild et al., 2018). In addition to regulating sleep and wakefulness, cholinergic and monoaminergic neurons are essential for learning, motivation, attention, reward, mood and locomotory behaviours (Eban-Rothschild et al., 2018). Hence, a sustaining activity of these wakefulness-promoting neuronal populations is essential for maintaining adaptive wakefulness.

3.3 Active and quiet state of wakefulness

Activity level in brain regions promoting wakefulness and arousal is not equal across the state. The differences may be broadly separated by dividing wakefulness into functionally active and quiet states. Active wakefulness represents a state of high arousal associated with

active behaviors, while quiet wakefulness represents a state of low arousal associated with quiescent behaviour. Such definition deviates somewhat between , as some defines the sub-states according to an aroused behaviour (e.g. Kang et al., 2017) and others use degree of physical activity researchers (e.g. Maloney et al., 1997). Some behaviours, such as grooming, are physically active behaviour but reflect a more offline state of the brain (Buzsáki, 2015). Other behaviours may be physically quiet behaviour but reflect an active brain state, such as freezing (Furlong et al., 2009) and ruminating (Pedersen et al., 2011).

Ideally, the state of wakefulness would be determined by a measurable cluster of behaviours and brain activity which are associated with activity or quiescence, but such a method does not currently exist (McGinley et al., 2015). Commonly used identification of active wakefulness and quiet wakefulness is based on different measures such as locomotor activity, activity of the whiskers, certain brain oscillations measured by EEG, muscle tonus measured by EMG, behavioural pattern, or the eye's pupillary diameter. Notably, all the mentioned measures indirect (correlates of) heightened arousal (McGinley et al., 2015). Consequently, the following sections relies on measures that indirectly relate to the construct of state-based separation of wakefulness.

3.4 Hypocretin signalling – a key stabilizer of arousal

Since the discovery of two hypocretin (orexin) neuropeptides in lateral hypothalamus in 1998 (De Lecea et al., 1998; Sakurai et al., 1998), studies have placed this system as a key integrator of homeostatic functions as an arousal stabilizer. Through the release of the neuropeptides hypocretin-1 and hypocretin-2, hypocretin neurons play an essential role in wakefulness regulation. These neurons interact with the major neurotransmitter networks in combining information that determines the arousal state, including the basal forebrain, tuberomammillary nucleus, cerebral cortex, locus coreleus and dorsal raphe nuclei, regions

involved in motivational, emotional, autonomic, and motor regulation (Alexandre et al., 2013; Peyron et al., 1998).

The data supporting the central role of hypocretin as an arousal stabilizer comes from studies in rats and mice showing that hypocretin neurons display a maximal firing (~8Hz) during active behaviours such as exploration and foraging (Mileykovskiy et al., 2005). Firing rate also correlates with higher EMG tonus and locomotory activity (Lee et al., 2005). Hypocretin neurons display less firing activity during automatic behaviours such as grooming, eating (~5Hz), and drowsy or quiet wakefulness (~1Hz). During NREM and REM sleep firing almost ceases (Lee et al., 2005; Mileykovskiy et al., 2005; Takahashi et al., 2008). In the human brain, a study using microdialysis reported higher levels of hypocretin-1 in the amygdalae during social interactions, and lower levels during eating behaviour and quiet wakefulness preceding sleep (Blouin et al., 2013).

Further evidence of the role of hypocretin in regulation of wakefulness comes from narcoleptic patients and rodent narcolepsy models. Narcoleptic patients with cataplexy is found to have lower cerebrospinal fluid levels of hypocretin-1 (Heier et al., 2007). The impaired hypocretin neuronal functioning is associated with impaired sustained attention, increased sleepiness, and sleep attacks during wakefulness (Naumann et al., 2006). Similar deficits are observed in mice models of narcolepsy when activity of the hypocretin is inhibited. The hypocretin neurons promote stable levels of arousal, as well as maintaining alertness for expression of motivated behaviours (Chemelli et al., 1999; Tsujino & Sakurai, 2013; A. Vassalli & P. Franken, 2017).

3.5 Electrophysiological characteristics of active and quiet wakefulness

Active and quiet wakefulness are also characterized by differences in neuronal brain activity, measured by EEG and/or local field potential recording (LFP). Navigating the

research field of EEG/LFP is difficult, since each author may define the range of the frequency bands differently. Also, measures from different scalp locations, depths of the electrodes and settings of recording, like sampling frequency, filtering of signals etc. give a variety of parameters that differ. In addition, since electrical signals can originate from far away the location of the electrode, and the EEG and LFPs only relays two-dimensional information (Kajikawa & Schroeder, 2011; Olejniczak, 2006). This means that the spatial precision is limited and interpretation of the origin of these signals is not possible. Despite these limitations, information from EEG and LFP offer a great advantage by enabling recording of electrical brain activity concurrently with behavior. By analyzing the spectral power in frequency bands of EEG and LFPs, several differences in brain activity between active and quiet wakefulness have been revealed.

3.5.1 Delta activity (~1-4 Hz)

Delta activity (or slow wave activity; <2Hz) dominates NREM sleep (Silber et al., 2007). These slow oscillations are also present during quiet wakefulness, but the amplitude is smaller (Grønli et al., 2016). Interestingly, the power of the SWA during quiet wakefulness increases with extended time in wakefulness, whilst SWA activity in active wake is little changed (Grønli et al., 2016; Vyazovskiy & Tobler, 2005).

3.5.1 Theta activity (~4-9 Hz)

Higher frequency theta activity (~7-9Hz) correlates with active wakefulness, and activity in this band is increased through activity inducing sleep deprivation methods in rats (Grønli et al., 2016; Vyazovskiy & Tobler, 2005). As wakefulness is extended however, the activity in the lower theta range (~5-7 Hz) is increased, most prominently in quiet wakefulness (Grønli et al., 2016; Vyazovskiy & Tobler, 2005). Frequency range with a mix of

delta and low theta (3-6 Hz) is found to increase during quiescent behavior such as grooming and being immobile in freely behaving rats. Frequency range in the higher range of theta and low alpha (6-10Hz) is found to increase during active behaviors such as rearing and moving (Neckelmann & Ursin, 1993; Young & McNaughton, 2008).

Increasing cortical arousal using glutamatergic stimulation of the basal forebrain has been reported to increase the power of the higher theta (7-9 Hz) and decrease activity both in the lower theta (5-7 Hz) band and SWA (Wigren et al., 2009). Interestingly, an increase in the ratio high to low theta (7-9Hz/5-7Hz) correlates with a high EMG-activity during sleep deprivation (Wigren et al., 2009). Thus, a ratio of high-to-low theta is increased with active physical behavior and decreased during quiescent behaviors like grooming. For this reason, it may be an appropriate measure for separating active and quiet wakefulness when physical activity levels are high.

3.5.3 Alpha activity (~10-15 Hz)

Alpha activity is the frequency band that defines human wakefulness when wake and sleep is scored based on the AASM manual. Alpha rhythm is present globally across cortices but shows the highest amplitude over the occipital cortex when subjects are awake with their eyes closed (AASM manual). Alpha activity is pronounced during higher arousal states of wakefulness, and attenuated by relaxing and drowsiness (Brown et al., 2012).

3.5.4 Beta (~15-35Hz) and gamma activity (~35-150Hz)

Beta activity and gamma activity are prominent during active and quiet wakefulness in humans. The power of these frequencies is found to be enhanced in response to presentation of sensory stimuli (Brown et al., 2012). The beta activity present in both active and quiet

wakefulness may reflect different neurobiological processes. In mice, active wake (by stimulation of whiskers and gentle handling) has been reported to elevate both beta and gamma (80-90 Hz) activity in frontal cortex (EEG) and in somatosensory cortex (LFP), indicative of cognitive processing. In quiet wake (recovery from the stimulation), beta activity paralleled delta and theta activity in tracking sleep need (Grønli et al., 2016).

The activity in the gamma band may also be dependent on the behavioral state of the animal. During movement, eating, the power in 30.5-58.0 Hz is reported to be enhanced, whilst grooming, attentive waking and quiet waking display a lower power in these frequencies (Maloney et al., 1997).

Another aspect of the functional significance of high gamma activity is that it may depend on the presence of theta activity. During active behaviors such as exploration, rearing, sniffing and active sleep (REM sleep), LFP recordings in hippocampus show dominance of high frequency theta (6–10 Hz) oscillations. During quiescent behavior, such as eating, grooming, immobility, and during quiet sleep (NREM sleep), large amplitude irregular activity or "sharp wave ripples" (SPW-Rs). SPW-Rs consists of large amplitude sharp waves(~5-15Hz), followed by ripple activity (110-200 Hz) dominate hippocampal LFP (Buzsáki, 2015). Gamma (30-120 Hz) activity is present in both theta dominated and SPW-Rs dominated behavior, regardless of the different states of arousal. Similar events have also been reported when LFP is recorded from the piriform cortex, amygdala, olfactory cortex, neocortex and thalamus (Buzsáki, 2015).

The increase in the lower frequency gamma (~20-50 Hz) increase quiet wakefulness may be an artefact of the high concentration of SPW-R as the frequencies overlap to some extent. Overlapping SPW-Rs have induce an increase in low gamma power), while non-overlapping SPW-Rs attenuate the power in this frequency range (Oliva et al., 2018). In sleep, the power

in low gamma frequency is significantly lower than in wakefulness (Carr et al., 2012; Oliva et al., 2018).

Thus, the activity in the gamma band might reflect both active (in particular) and quiet behavior, but the function of gamma activity is still unclear.

4.1 Adaptive wakefulness capacity and active wakefulness

The differences in how active and quiet wakefulness are regulated are due to the distinct differences in adaptive value. For survival, both predator and prey rely on active wakefulness (Löw et al., 2008). For the sexual reproduction (Schober & Pfaff, 2007), to explore (McGinley et al., 2015), being able to sustain attention or being alert to the environment (Alexandre et al., 2013) – and more - depend on a higher state of arousal. In sum, active wakefulness can be considered essential for the animal to survive and to reproduce.

Quiet wakefulness is incompatible with reproduction but is still adaptive for survival. During quiet wakefulness, the animal may react to environmental stimuli and at the same time use less energy than in active wakefulness (DiNuzzo & Nedergaard, 2017). Through inactivity, the animal also diminishes detectability from predators, favouring survival and reduces risk of injury. It is also involved in restitution from muscle fatigue. (Siegel, 2009; Xia & Law, 2008; Zoccoli & Amici, 2020).

As many of the above-mentioned functions of quiet wakefulness might be achieved more effectively in sleep (Del Bo et al., 1982), at lower energetic costs (DiNuzzo & Nedergaard, 2017), the adaptive value of quiet wakefulness is tied to the ability to respond to the environment. A quietly wakeful animal relies on switching towards active wakefulness if a threat is detected or a mating opportunity is nearby. Thus, if the capability to enter active

wakefulness is hampered, by low cognitive functioning, the adaptive value of both active and quiet wakefulness is lowered.

Lima and colleagues (2005) suggest that the state of quiet wakefulness is adaptive when the likelihood of a predatory encounter is high and the animal respond to predators effectively. Supporting this view is a study showing that presence of a simulated predator delayed sleep in wild caught *Ratticus norvegicus* (Lesku et al., 2008). Taken together, when the likelihood of an effective waking state is low, sleep may represent a more adaptive state for the animal.

Similar logic applies to the likelihood of available food and water, mating partner, or other vital for the specie. If available, then a higher responsiveness and higher processing of sensory information during quiet wakefulness will allow for adequate response, as in contrast if the specie was asleep. Little research has investigated how sleep is affected by availability of vital resourcers for survival and reproduction. One study in the sand piper found suppression of sleep when the sex drive was high (during mating season) (Lesku et al., 2012).

4.2 Need-based regulation of wakefulness

Whether an individual enters active wakefulness mainly depends on if there is a need to be in that state. Safety from predator activity and sexual drive are already mentioned, in addition, hunger and thirst serve as a powerful wake-promoting signal for increasing locomotory activity and level of arousal (Borbély, 1977; Danguir & Nicolaidis, 1979; Dewasmes et al., 1989). Animals with offspring also need to take care of their young (Eban-Rothschild et al., 2018).

4.3 Environmental regulation of wakefulness

Since the effectiveness of active wakefulness is to a large extent determined by the individual's environment, it is no wonder that environmental stimuli directly influence the regulation of being aroused. A night-active species is adapted to being awake during darkness, and to be less active during light-hours. Rats is one example. They have a poor visual acuity, in other words they are very near-sighted, and rely to a great extent on whiskers and nose for navigation in the night (Burn, 2008). During daytime, the rat is less able to exploit light information than it's resource-competitors or predators. Humans on the other hand, rely on visual information to a much larger extent for their adaptive behaviours. It is well established that the light condition directly alter state of wakefulness; darkness is the arousal signal for nocturnal animals, as light is the arousal signal for diurnal animals (Jha et al., 2021).

4.4 Circadian regulation of wakefulness

Circadian oscillators are other important factors that regulate endogenously the efficacy and state of arousal. The observation that circadian activity patterns persist without light as a wake-signal (constant darkness) in humans (Aschoff, 1965) has been observed in all species described so far, like fruit flies (Dubowy & Sehgal, 2017), zebrafish (Elbaz et al., 2013; Hurd et al., 1998), zebra finches (Wang et al., 2012), rats and mice (Borbély & Neuhaus, 1978). These endogenous oscillators are entrained to time cues in the environment referred to as *zeitgebers*. Diverse cues such as light (Gooley et al., 2003; Whitmore et al., 2000), temperature (Buhr et al., 2010), stress (Tahara et al., 2017), exercise (Yamanaka et al., 2006), food availability (Stephan, 2002) and social interactions (Bloch et al., 2013; Fuchikawa et al., 2016) serve as zeitgebers, where light has the strongest influence to increase accuracy of prediction.

The detection of phasic circadian patterns of activity may either be attributed to intrinsic circadian oscillators or determined by what environment they are studied in. For the model

fruit fly *Drospholia melongaster*, the existence of peaks in locomotory activity during the dusk and dawn has been attributed to two different circadian oscillators due to persisting in darkness or dim red-light conditions (Helfrich-Förster, 2000; Helfrich & Engelmann, 1983; Yoshii et al., 2004). An additional peak in locomotory is present during the afternoon under more naturalistic settings (Green et al., 2015), This illustrates that circadian rhythms must be studied in constant environments if the rhythms are to be considered intrinsic.

Circadian rhythms divided by regular cycles of light and darkness has been one of the most regular and predictable occurrences in life, since life first originated on Earth about 3.8 biollion years ago. For more than 700 million years, organisms have had the tools to detect, predict and adapt to these changes (Foster & Kreitzman, 2017). Consequently, circadian rhythms increase the adaptive value of the wakefulnesss. As such, changes in the environment and internal oscillators offer powerful regulation of adaptive wakefulness. However, the understanding of the efficiency of adaptive wakefulness is still insufficient.

4.5 Motivational drive of wakefulness

The motivational drive to take certain actions is found to be impaired by longer time spent in wakefulness. An acute reduction in performance is described already after 8-10 hours of wakefulness and such impaired effectiveness of waking behaviour is being explained by reduction in functional abnormalities in the brain which reduce the motivational drive. Its insufficiency stems from the fact that sleep deprived animals exhibit reduced performance and alertness despite peaks in circadian modulation (Doran et al., 2001; Monk et al., 1985), constant environmental conditions (Cain et al., 2011) and motivational incentives to perform (Hsieh et al., 2010).

The internal system to stimulate motivation, encourage alertness and behaviour is also a crucial factor for the adaptive value of wakefulness. Sleep strongly affects the motivational

state, in humans and animals. To understand what may cause this deficiency we must consider what functions of sleep that may allow its restoration.

6.1 Functions of sleep

Researchers generally agree that not one, but several physiological roles are served in sleep (Zielinski et al., 2016). In the recent decades, multiple hypothesis about the physiological roles of sleep has been purported. These include brain development (Mirmiran et al., 1983), memory consolidation (Abel et al., 2013) , immune response (Zielinski & Krueger, 2011), energy conservation (Berger & Phillips, 1995), anti-predatory inactivity (Siegel, 2009) and more. While these hypotheses highlight possible benefits of sleep, they may not explain how adaptive wake capacity is restored by sleep.

6.2 Perspectives on adaptive wake capacity explained by sleep

An unresolved question is whether sleep is regulated to compensate for a burden incurred by wakefulness, or whether it is the sleep regulation itself that incurs the performance related impairments in wakefulness. The former case suggests that the impairments is a consequence of some factors outside of sleep regulation. In the latter case, it is assumed that sleep is so beneficial compared to wakefulness that actively incurring performance impairments in extended wakefulness to achieve more of sleep increases the net reproductive success.

These perspectives are not mutually exclusive. The mechanisms of sleep regulation could very well incur performance impairments that are smaller than the impairments incurred in their absence. In other words, the restorative regulatory properties from sleep can be an important benefit for vital cognitive skills, including performance, that makes incurring impairments in extended wakefulness adaptive. Several perspectives on sleep regulation are compatible with this view.

7.1 Sleep regulation perspectives

7.1.1 The two process model

After extending time in wakefulness or being more active during the time of wakefulness, the subsequent sleep episode has an increase in the intensity of the deep sleep (stage N3 or SWS) and (to some extent) longer time in sleep. This regulatory trait suggests that sleep is under homeostatic regulation, and that an equilibrium has been disturbed by the extended time in wake or the intensity of wake. By increasing the length and intensity of sleep, the equilibrium may be restored.

The homeostatic compensation of sleep loss is non-linear. Relatively low compensation in sleep length can be illustrated by a subject who stayed awake for 264 hours and the recovery sleep was only 14.4 hours (Gulevich et al., 1966). A non-linear explanation of sleep regulation was addressed in Boberly's two-process model of sleep regulation (1982). He proposed that homeostatic sleep pressure (labeled "process S") increased non-linearly as a function of time in wakefulness. Further, he suggested that dissipation of the sleep pressure was also a function of the intensity of sleep (measured by SWA). A second process was also proposed ("process C") to account for the periodic variation sleepiness propensity, subjective fatigue, sleepiness, and alertness levels (Daan et al., 1984; Åkerstedt & Folkard, 1997). These periodic variations correspond roughly to the circadian rhythm of alertness (Borbély, 1982).

Laboratory, clinical and field studies in different populations and species have to date not been able to falsify this model (Borbely et al., 2016). Typically, sleep deprived subjects show performance detriments during the sleep privation period, increased sleep pressure by shortened latency to sleep and increased time in sleep, and increased sleep intensity by a higher slow wave activity in subsequent NREM sleep (D. Dijk et al., 1990; D. J. Dijk et al., 1990) .

Three decades of sleep research later, this model is still one of the most influential models of sleep regulation, and as mentioned above, no study has yet been able to falsify this model. However, the model has been extended in important aspects (Borbely et al., 2016). 1) process S is also influenced by the time of day (Vyazovskiy et al., 2007), at least when measured by EEG SWA. 2) The amplitude of process C is attenuated by increasing time awake(Borbely et al., 2016). 3) The SWA response is dependent on local wake intensity in the brain (Huber et al., 2004).

7.1.2 Local sleep pressure

A homeostatic response to local intensities of wakefulness is demonstrated in humans, (Huber et al., 2004) and rats (Vyazovskiy et al., 2011). More recently, the assumption that sleep pressure increases with the overall time in wakefulness has been challenged. In mice, mathematical modelling of process S suggested that the build-up of SWA during time in active wakefulness may be better prediction than hours of wakefulness (Vassalli & Franken). Moreover, time in active wakefulness (EEG-defined) predicts better such build-up than time in quiet wakefulness or total wakefulness. The model fit was also improved when the hypocretin secretion was impaired (silenced HCRT gene; Vassalli & Franken, 2017).

Together these findings suggest that the slow-wave activity previously attributed to sleep also occur during wakefulness. Moreover, a build-up of these slow oscillations is a marker of sleep pressure (process S), and the intensity of SWA during wake is highly dependent on time in active wakefulness and not on time in quiet wakefulness(Vassalli & Franken, 2017; Vyazovskiy & Tobler, 2005)

8.1 Functional perspectives on sleep homeostasis

Promising work has begun to identify the mechanisms behind the increase in SWA during wake and sleep. Three major concepts explain this phenomenon by differences in the brains' metabolic demand: by the means of synaptic homeostasis, energy depletion, and the waste clearing.

8.2 The synaptic homeostasis hypothesis

The synaptic homeostasis hypothesis (SHY) describes an increase in the homeostatic pressure during wake by synaptic potentiation, and the relief of sleep pressure during sleep due to synaptic downscaling (Tononi & Cirelli, 2006). A persistent synaptic potentiation (long-term synaptic potentiation, LTP) is a persistent increase in synaptic efficacy favoring the communication between neurons and learning. To maintain such potentiating by being aroused or in active wakefulness is metabolically demanding. When the synaptic weights increase, being awake and aroused becomes more demanding. Reasonably, there is a need to downscale for the wakefulness not to become too demanding. A slow wave (like 1Hz) roughly corresponds to the optimal frequency for induction of long-term depression (LTD ;Léger et al., 2018). In terms of adaptive wakefulness capacity, SHY could very well explain the cost of long-term wakefulness as a function of an increased metabolic demand of the late-phase wakefulness (Vyazovskiy et al., 2008). Ultimately, SHY is compatible with an increase in global SWA during time in active wakefulness, since behaviors associated with active wakefulness increase the long-term potentiation of the neuronal synapses (Tononi & Cirelli, 2006).

8.3 The energy depletion hypothesis

The energy depletion hypothesis suggests that the demand of being awake has a taxing effect on the capacity of neurons to signal effective and correct, due to depletion of the energy stores. The argument of this hypothesis is simple and effective: Anabolic processes are

needed to build (complex) molecules for signaling activity, and this energy use is higher than energy release (catabolism). If the energy required to do cellular work during wakefulness is used at a greater rate than it is synthesized, wakefulness capacity is reduced.

One organic compound for this process is glucose, a food derived molecule that can be used to produce adenosine triphosphate (ATP), the primary energy currency of the cell. When ATP us expended at increasing rates, it is considered an increase in metabolic demand. Calculation estimates suggest neuronal signalling activity is the most metabolically demanding process in the brain (Attwell & Laughlin, 2001). During periods of high metabolic demand, or when the supply of glucose is low, the brain may utilize its only stored reserve of energy; glycogen (Sofroniew & Vinters, 2010). These stores may be depleted during extended wakefulness (Kong et al., 2002), and therefore, serve as limiting factors to adaptive wakefulness capacity.

Adenosine is another candidate molecule for the energy depletion hypothesis. When ATP production is limited locally in the basal forebrain of rats, the extracellular products of adenosine and lactate has been shown to increase (Kalinchuk et al., 2003). It also increases during extended wakefulness and is reduced during sleep in important wakefulness sustaining brain regions. Relatedly, high levels of extracellular adenosine acts to inhibit the arousal promoting activity of basal forebrain and hypocretin neurons (Liu & Gao, 2007; Porkka-Heiskanen et al., 1997).

Neuronal signaling activity is needed for all cognitive processes. If the energy to sustain it is depleted, it would undoubtedly impact performance. In this regard, the hypothesis that brain energy is depleted during extended wakefulness is clearly relevant for explaining the impairments in performance after extended wakefulness if correct.

8.4 The waste clearing hypothesis

The importance of waste clearing for sleep homeostasis is an increasingly documented hypothesis (Hauglund et al., 2020; Kang et al., 2009). Like the energy depletion hypothesis, the high energetic demand of wakefulness is recognized to be culprit. Catabolic processes generate numerous metabolites which hamper effective neuronal signaling or in the worst case accumulate as placks (Kang et al., 2009). The clearing of metabolites from the cellular environment is important both for continued activity of the cell, and to avoid cellular death. This is in part achieved by the glymphatic system. The glymphatic system is a glial regulated waste-clearance pathway. It ”flushes” out extracellular metabolic waste through a mix of interstitial and cerebrospinal fluid, which exits through venous drainage (Hauglund et al., 2020).

Theoretically, if cellular waste is cleared at a greater rate than it is produced, waste would not be a concern for adaptive wakefulness capacity. However, several lines of research suggest that waste products accumulate extracellularly during wakefulness (Hauglund et al., 2020). A decreased noradrenergic tone during NREM sleep allows for an increase in the interstitial space permitting the glymphatic system to more efficiently clear waste from brain tissue (Xie et al., 2013). Peptides like tau and amyloid β, and others have been shown to be cleared at a greater rate during NREM sleep than wakefulness (Hauglund et al., 2020). More controversially, is lactate, one of the metabolites produced by cells when food is catabolized into energy. Since active clearance of lactate occurs by the glymphatic system, this molecule has been hypothesized to be cleared as a safe shuttle to remove excess carbon (Lundgaard et al., 2017).

In terms of adaptive wakefulness capacity, this perspective suggests that extending wake beyond a certain time (or use) range increases the global (or local) concentration of waste to detrimental levels. Accumulations may limit adaptive wakefulness. The waste clearance hypothesis is also plausibly linked to the negative association between density per

surface area (greater surface area more efficient waste exchange) and length of wakefulness in mammals.

9.1 High metabolic demand limits restorative processes

Both the energy depletion and the waste clearing hypothesis are linked with activity in astroglia cells. Astroglia are about 5 times more numerous in the brain than neurons (Sofroniew & Vinters, 2010). They serve a wide range of supportive roles in the brain. This includes supply of oxygen to neurons through regulation of blood flow, maintaining synapse transmission homeostasis, and metabolic waste clearance through the glymphatic system (Hauglund et al., 2020; Sofroniew & Vinters, 2010). They also play a role in resupply of vital molecular building blocks to the neurons they support (Sofroniew & Vinters, 2010). Finally, astrocytes store glycogen, which enables them to support high signaling activity during periods of low blood sugar, or high firing rates of neurons, although for a limited duration (Sofroniew & Vinters, 2010).

A common idea among the above hypotheses on synaptic downscaling, the metabolic energy restoration and waste clearing is that the reduction in signaling activity is necessary to reverse the negative burden of extended wakefulness. Astrocytic regulation is involved in the regulation of how much energy is expended in signaling activity. Release of Ca^{2+} into the extracellular space promotes the release of signaling substances. Astrocytes cells increase their signaling of Ca^{2+} activity in response to cholinergic(Takata et al., 2011) and noradrenergic (Paukert et al., 2014). A recent study demonstrated that during active wakefulness astroglia Ca^{2+} release was much greater than during more quiet wakefulness, and further reduced during sleep (Bojarskaite et al., 2020). Interestingly, astrocytic Ca^{2+} signaling has also been implicated in increase of neuronal “down states” of neurons, a process that is thought to generate SWA in quiet wakefulness and sleep (Szabó et al., 2017).

It is possible that the down states associated with SWA are necessary to perform efficient waste clearing activity and restore metabolic brain energy. During high neuronal signaling activity in active wakefulness, the rate of oxidative phosphorylation is decreased (DiNuzzo & Nedergaard, 2017). Instead the cells increase the rate of aerobic glycolysis (DiNuzzo & Nedergaard, 2017), a process that may only generate a fraction of the ATP of oxidative phosphorylation (Dienel, 2019). Active metabolic waste clearing and restoration of metabolic brain energy (e.g. glycogen) are both processes that depend on ATP. Thus, upregulation of these processes while the neurons are engaged in signaling activity likely requires more glucose than during low signaling activity. Ideally then, the cells downregulate signaling activity before increasing active metabolic waste clearing, and brain energy restoration.

Combined, these hypotheses also provide plausible explanations to why adaptive wake capacity is time-and use limited. A metabolic demand that increases across wakefulness (due to increases in synaptic weights), will very well increase the impairment of performance (and negative health in general) as the need for depletion of waste products exceeds the physiological resources. Both processes are plausibly linked to the need to reduce aerobic glycolysis to increase their efficiency.

9.2 Lactate, a marker of metabolic demand

Increasing extracellular levels of lactate are associated with an increase in aerobic glycolysis (Dienel, 2019). Across wakefulness and REM sleep extracellular levels of lactate increases, and in NREM sleep it decreases (Rempe & Wisor, 2015). The increase in lactate during wakefulness may be linked to more active wakefulness (Grønli et al., 2016; Wigren et al., 2009). .

Intriguingly, markers of sleep homeostasis, such as SWA, theta and beta oscillations during quiet wakefulness are suggested to play a role in the reduction in lactate during quiet

wakefulness. Lactate dynamics in mice has been model using a five-vigilance state model, which included intermediary (no change in lactate), active (increased lactate) and quiet wakefulness (decreased lactate), REM (increased lacatate) and NREM (decreased lactate) as states (Grønli et al., 2016). Grønli and colleagues 2016 demonstrated that lactate concentration in the cerebral cortex of mice was more accurately predicted using five-state model, than using a three-state model (wakefulness increase, NREM sleep decrease and REM sleep decrease). The reported decrease of lactate during quiet wakefulness episodes was due to transitions of quiet wakefulness into sleep, but the low lactate concentration was found to be present during the quiet wake. Moreover, when the quiet wakefulness was characterized by beta oscillations, this state of wakefulness was permissive to metabolic and electrophysiological changes that occur in quiet sleep (NREM sleep). Relatedly, Wisor and colleagues (2013) demonstrated a reduction of extracellular lactate in pyramidal cells of mice by optogenetic induction of slow waves (1Hz) during wakefulness.

10.1 Restoration by quiet wakefulness

On the basis of the literature presented, I propose that adaptive wakefulness capacity is time and use limited due to availability of energetic- and synaptic resources, and accumulation of metabolic waste. High metabolic demand during active wakefulness reduces the capacity of adaptive wakefulness, whilst an enhanced glucose utilization (low metabolic demand) during quiet wakefulness and quiet sleep (NREM sleep) allow the capacity of adaptive wakefulness to be restored (figure 1).

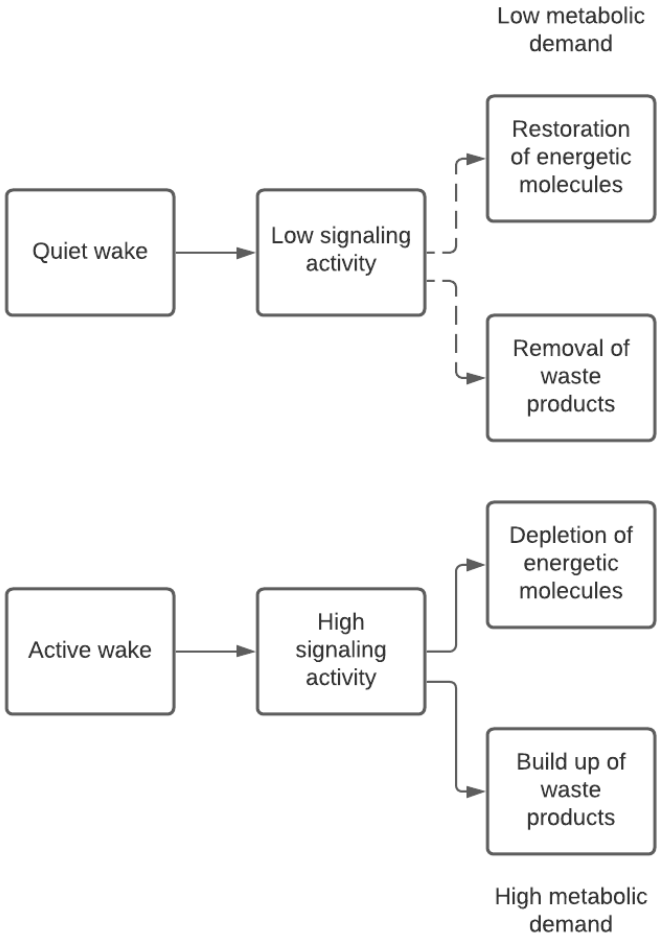

Figure 1: The metabolic demand of active and quiet wakefulness.

High neuronal signalling activity during active wakefulness is hypothesized to cause a build-up of metabolic waste products, and to drain brain metabolic energy. The build-up of waste substances is associated to the rate of reactions, and to slowness of the efficiency of waste clearance (due to noradrenergic tone or low availability of energy reserves). In contrast, the lower neuronal signalling activity during quiet wake generates less waste-products, the noradrenergic tone is low which increases the efficacy of waste clearance. Dashed lines represent hypothetical relationships

This perspective is consistent with local sleep homeostasis. The model suggests that sleep-like homeostatic processes can occur during the state of being awake. Furthermore, it is hypothesized that this local process has global implications: if the concentration of metabolic waste and energy resources are depleted in the wake-promoting brain region (e.g. hypocretin

neurons, basal forebrain or locus coreleus), then the net effect in this/these neuronal population(s) should be an increase in a quiet state (quiet wakefulness or NREM sleep) of the animal. A reduced state of arousal involves both the local neuronal population and its' projection sites. If one local brain area displays a quiet state, this may have inhibiting effect on the state of active wakefulness as this behavior require a concert of wake-promoting signaling throughout the cortex (Poulet & Crochet, 2019; Vyazovskiy et al., 2011). An individuals' need for a quiet state should ideally be consolidated during periods of low activity or inactivity or automatic behavior, when the consequences on performance are less important. By being quietly awake, the individual maintains readiness to respond to its' surroundings. If quiet wakefulness is effective in restoring adaptive wakefulness capacity, we may expect that wakefulness is regulated homeostatically.

10.2 Homeostatic regulation of wakefulness

Modell and Colleagues (2015) outline five critical components that are part of any homeostatically regulated system. The system must contain, 1) a set point, 2) a sensor 3) an error detector, 4) a control system, and 5) effectors.

1) The set point is a value, or normal range of values, with which the value of the regulated variable is compared
2) A sensor is a receptor capable of measuring the regulated variable
3) An error detector compares the signal of the sensor with the set point
4) The controller interprets the error signal, and determines the value of the outputs
5) Effectors are the elements whose actions changes the value of the regulated variable.

In the following section, I argue that all these components are involved in regulating wakefulness. To explicitly state the falsification criteria of this model, I use hypocretin

regulation as a proof of concept. This does not mean that hypocretin is the only mechanism involved in regulation of homeostatic wakefulness, but without explicitly stating a mechanism, the model cannot be falsified. The model is outlined in Figure 2.

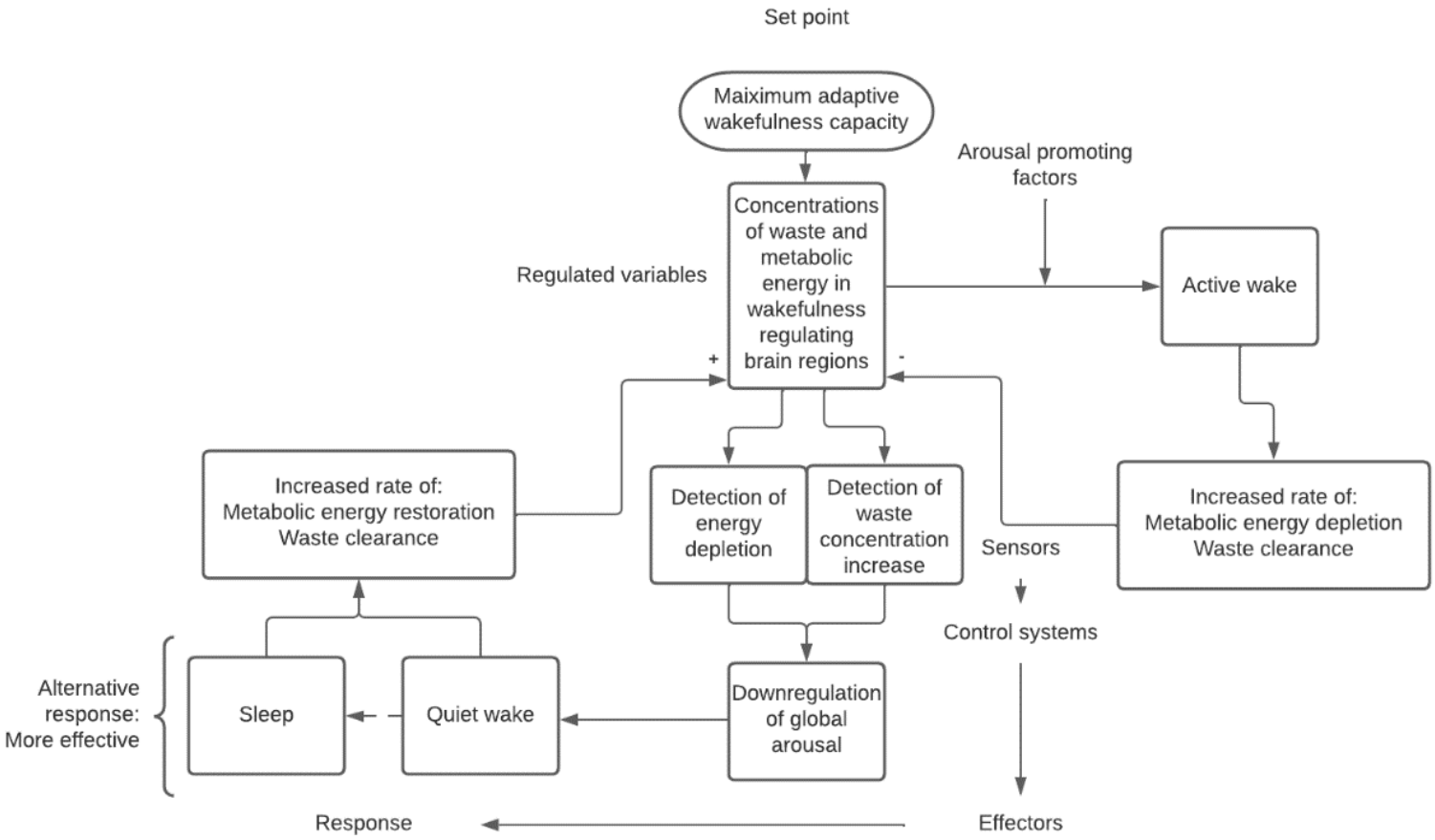

Figure 2. A model of homeostatic wakefulness regulation.

Arousal promoting factors include drives, circadian regulation, and environmental stimuli. These causes the animal to enter an active wakefulness state. In active wakefulness, metabolic brain energy necessary for optimal performance are decreased, and waste concentrations associated with negative health outcomes are increased extracellularly over time. This decreases the adaptive value of wakefulness. As active wakefulness progresses, sensors detect the reduction of adaptive wakefulness capacity. To increase the capacity, arousal is inhibited. If this inhibitory mechanism is stronger than the arousal promoting mechanisms, the animal may enter

a state of quiet wakefulness. Whether the animal enters sleep from quiet wakefulness depends on the current adaptive value of wakefulness: How likely it is that responses to opportunities and threats are effective. If this is high, quiet wakefulness is preferred to sleep. However, sleep is a more effective response to the decreased capacity, due to lower metabolic demands. In quiet wakefulness or sleep states, metabolic waste is cleared, and metabolic energy is restored. This increases the adaptive wakefulness capacity. With higher adaptive wakefulness capacity, the animal may stay awake longer.

The regulated variable in the model of homeostatic regulation of wakefulness is brain metabolic energy and waste concentration. When reserves of limited brain metabolic energy high, performance may be sustained for longer periods of time. In addition, when waste concentrations are low, the animal may sustain longer periods of wakefulness without negative health outcomes. Waste molecules associated with increased concentration during extended wakefulness include Aβ, tau-proteins (8.4), and possibly lactate (8.4,9.1), while astrocytic glycogen stores are examples of limited brain metabolic energy (8.3). When the concentration of waste and reserves of brain are at their highest, the animals are considered to have maximum capacity of adaptive wakefulness. Since this capacity is thought to be essential for survival and reproduction, it may be considered the set point of the model.

When arousal promoting factors, such as needs (4.1), environmental stimuli (4.2), circadian regulation (4.3) or motivational drives (4.4) promote arousal, the animal may enter active wakefulness. In active wakefulness, the signaling activity is higher(DiNuzzo & Nedergaard, 2017). Signaling activity is metabolically demanding, increasing the production of metabolic waste- and expending of metabolic energy. Waste clearing and energy restoration is less effective during high signaling activity due to decreased oxidative phosphorylation (9.1-9.2). Instead of increasing waste clearing activity, it is likely that reducing concentrations of waste and increasing metabolic energy rely on reducing signaling activity. Thus, active wakefulness decreases the adaptive wakefulness capacity. This claim

may be falsified if it is shown that net waste clearing activity occurs or that brain energy stores are replenished during active wakefulness.

In support of this view, high hypocretin levels have been implicated in maintaining active wakefulness (2.6) and are associated with increased concentration of Aβ (Kang et al., 2009). As mentioned, lactate levels may also increase increase as a function of active wakefulness (Grønli et al., 2016; Wigren et al., 2009). Finally, glycogen stores are examples of brain metabolic energy that reportedly decreases as a function of wakefulness (Benington & Heller, 1995).

When concentration of metabolic waste increase and metabolic energy depleted, adaptive wakefulness capacity is reduced. While capacity is decreased, certain molecules such as adenosine increases in concentration (Porkka-Heiskanen & Kalinchuk, 2011). When they are low, they signal that the capacity is high, and when they are high, they signal that the capacity is low. As they allow assessing whether the capacity of adaptive wakefulness is decreased, these molecules are examples of error detectors in the homeostasis. Consequently, the receptors that detect adenosine levels (e.g A1 adenosine receptors) may act as sensors of decreased wakefulness capacity. This claim may be falsified if it shown that adenosine does not increase along with metabolic waste- and brain energy reserve depletion.

When A1 adenosine receptors detect an increase in concentration of adenosine, they may act to decrease activity. If the decrease in activity occurs in wakefulness promoting regions, such as the basal forebrain or hypocretin neurons in the hypothalamus, this also inhibits arousal (Porkka-Heiskanen & Kalinchuk, 2011). Since wakefulness relies on sustained activity in these brain regions (2.5), and the reduction of their activity mediates a reduction in signaling activity, they may be considered control systems of the homeostasis. As lower hypocretin levels reduce the signaling activity they may act as effectors in the model.

This claim may be falsified if it is shown that adenosine does not decrease the firing rate of hypocretin neurons, or hypocretin is shown to not impact signaling activity.

As activity in wakefulness-promoting brain regions are inhibited, the animal may enter a state of quiet wakefulness or sleep. Whether an animal responds to the homeostatic pressure by entering quiet wakefulness or sleep likely depends on the prospective effectiveness of responses in active wakefulness (3.1, 4.1-4.3). This is supported by the association between hypocretin neurons and reduced firing rates during quiet wakefulness and sleep (2.6). During the state of quiet wakefulness and sleep, the signaling activity is reduced. This allows restoration of metabolic brain energy and removal of metabolic waste products (9.2, 10.1). As time in this state progresses, adenosine levels are decreased. These claims may be falsified by showing that brain metabolic energy does not increase, and adenosine or metabolic waste levels do not decrease with time in quiet wakefulness.

Models are usually more useful in generating hypotheses than in accurately representing a phenomenon. Instead of testing the accuracy of the model, a discovery-oriented approach tests hypothesis that are predicted based on models (Oberauer & Lewandowsky, 2019). This approach is useful when the prediction itself is interesting. The core prediction of this model is that quiet wakefulness is homeostatically regulated to increase adaptive wakefulness capacity. This could be interesting in the light of, and without the light of the mechanisms proposed here.

10.3 Testing the prediction that quiet wakefulness is homeostatically upregulated to increase adaptive wakefulness capacity

Interesting findings could indicate that a homeostatic regulation of the waking states occurs. In two studies of simulated night shift work, rats forced to be active (simulating work) in their inactive/resting phase (rest workers) were compared to rats being active during their

active phase (active workers). Active workers had to 'work' for 8 hours centered in the 12h light phase, starting 2 hours after light onset. Rest workers 'worked' 8 hours centered in their 12 h dark phase, with onset 2 hours after lights off. Across several days of shift work (3 or 4 consecutive shifts), all rats slept less than their own baseline. Rest workers sleep less than active workers during the shift work period. However, rest workers did not display an increase in the homeostatic sleep pressure measured by SWA in the EEG. Instead, the rest workers displayed marked increases in of slow wave sleep like EEG oscillations (SWA) in quiet wakefulness (Grønli et al., 2017; Marti et al., 2020). It remains plausible that the rest workers partially compensated for sleep loss during the state of wakefulness by homeostatic regulation during quiet wake. Another possibility is of course that the rest workers did not compensate for the sleep loss during the shift work period. An incomplete restoration of metabolites, waste or metabolic energy reserves associated with the longer time awake is likely, since the rest workers exhibited an impaired spatial performance in a Morris water test at the end of a three days shift work schedule (Marti et al., 2020).

These findings are encouraging from my perspective on modelling effective adaptive wakefulness and if a quiet state of wakefulness/arousal is homeostatic response happening during wake (and not solely during sleep). These studies sub-staged all epochs of wakefulness into either active, intermediary or quiet wakefulness, based on which 33th percentile of the EMG signal they belong to (Grønli et al., 2017). This algorithm separate epochs of inactivity from high locomotory activity, and time in quiet wakefulness is defined on the base of the total epochs scored as awake during a chosen interval (typically 24 hour).

As this score is percentile-based, it increases with the interval length of which it is measured. For instance, if quiet wakefulness is scored as the 33th lowest percentile EMG of 1000 epochs of wakefulness; the amount of epochs scored as quiet wakefulness will be ~333.

If the wakefulness is scored by 1500 epochs, 500 epochs will be scored as quiet wakefulness. Thus, we cannot infer from this scoring method that adaptive wakefulness capacity is increased by an increase in quiet wakefulness. This limitation is redeemed when we have multiple days of recording, since baseline values can be used to determine the percentile score and be applied to the subsequent days.

Another consideration is that, when analyzing the laboratory rats' wakefulness, much of their time will be in brief wakefulness episodes (lasting <5 minutes; Simasko & Mukherjee, 2009). These episodes are less like the monophasic ~16-hour wakefulness of humans, and more like brief awakenings during the night. It is unlikely that much homeostatic pressure increases such a short span of time. The opposite suggestion, that it is decreased, has been supported by comparisons of SWA and SWS length after a brief wakefulness episode (Franzen et al., 2008). Thus, if the aim of analysis is to infer properties about human wakefulness, an important consideration regards whether to include these episodes in analysis as the same state.

A final consideration is that, as discussed, my definition of quiet wakefulness is not based on physical activity. Most studies use a physical activity definition of quiet wakefulness. Thus, I need to verify that arousal levels are low for my operationalization to be relevant for my model.

Research on sleep homeostasis is based on staging sleep into NREM sleep and REM sleep based on EEG and EMG criteria. This is an effective tool to measure intensity and the length of sleep homeostatic response. Research on wake homeostasis, however, as arguably, has no standardized method to stage wakefulness based on EEG and EMG criteria. Here, the question of whether wakefulness is homeostatically regulated, and if time in quiet wakefulness increases the capacity of adaptive wakefulness was approached using a hypothesis driven analysis of rat wakefulness in undisturbed conditions.

Methods

Hypothesis driven analysis

24-hours of electrophysiological data from two undisturbed and freely moving rats in a controlled 12:12 LD environment was examined.

During recording, the rats were situated in their home cage with food and water available *ad libitum*.

Ethical Approval

Protocols for use of rats were approved and registered by the Norwegian Animal Research Authority (Permit Number: H1 - 11321, H2 -– 2012463). The experiments were performed in accordance with Norwegian laws and regulations on the use of live animals in experimental conditions and the European Convention for The Protection of Vertebrae Animals and Other Scientific Purposes.

Animals and Housing

Baseline telemetric recordings from adult male rats (approximately 3 months of age) were used in this thesis. Data from two rats was used for the hypothesis driven approach (referred to as H1, Sprague-Dawley strain, nTac:SD, Taconic, Denmark) and data from nine more rats were used for the hypothesis testing approach (referred to as H2, Wistar strain, NTac:WH, Taconic, Denmark H1).

All rats had been subjected to similar housing conditions and surgery procedures (H1; Marti et al., 2020; H2; Grønli et al., 2017) In short, after arrival and acclimatization to laboratory conditions, the rats were group housed in individually ventilated cages (IVC) before surgery (IVC II; 480 × 375 × 210 mm, Tecniplast, Italy). After surgery the rats were single housed in IVC III cages (425 × 266 ×185 mm, Tecniplast, Italy). The cage ventilation

provided 75 air changes/h, temperature was maintained at 23 ± 1 °C, and humidity at 40% ± 1% throughout the experiment. Food (rat and mouse no. 1 (RM1), Special Diets Services, Witham, Essex, England) and water was available *ad libitum*.

The animals were kept on a 12:12 light-dark (LD) schedule, with gradual transitions from light to dark phase from 06:00-07:00 h and gradual transition from dark to light phase from 18:00-19:00 h. Zeitgeber time 0 (ZT0, lights on) was set to be at 07:00 and ZT12 (lights off) at 18:00 h. Mean light intensity in the light phase was 222 ± 112 lux.

Surgery

To obtain simultaneously electroencephalogram (EEG) and electromyogram (EMG), 4ET transmitters (Physiotel®; Data Sciences International) were surgically implanted. Antibiotic was given to the rats through drinking water up to three days before the surgery (trimethoprim, 0.16 mg/ml; sulfamethoxazole, 0.8 mg/ml; Bactrim, Roche; Basel, Switzerland). Each surgical intervention was performed under anesthesia; s.c. injection of fenatyl, (0.227 mg/kg, Hypnorm, Janssen Pharmaceuticals, Beerse, Belgium), fluanizone (8.8mg/kg, Dormicum, Roche) and midazolam (2.5mg/kg, Actavis, Teva Pharmaceuticals, Peta Tikva, Irsael) mixture.

EEG elecrodes were intracranial implanted frontal-parietal (FP, located at bregma: AP = 2.0 mm, ML = − 2.0 mm and lambda coordinates: AP = 2.0 mm, ML = 2.0 mm) and frontal-frontal (FF, located at bregma AP = 2.0 mm, ML = − 2.0 mm and lambda coordinates: AP = 2.0 mm, ML = 2.0 mm). Two sterile EMG electrodes were bilaterally and subcutaneously implanted in neck muscles in the dorsomedial lumbar region.

After surgery, Ringer's acetate solution (5 ml i.p, Baxter) was immediately given to compensate for fluid loss. The rats were given antibiotics in their drinking water for 2 days following surgery (trimethoprim, 0.16 mg/ml; sulfamethoxazole, 0.8 mg/ml; Bactrim, Roche),

and for 3 days an anti-imflammatory treatment (meloxicam; 5 mg/ml, subcutaneous., Metacam, Boehringer Ingelheim, Germany) and an analgesic (buprenorphine; 0.30 mg/ml, subcutaneous., Temgesic, Reckit & Benckiser; Slough, UK) were given one and two times per day respectively. A minimum of two weeks with daily care was provided for recovery (Moscardo & Rostello, 2010).

Experimental conditions

The chronically implanted rats were recorded undisturbed and freely moving for at least 24 hrs. All were single housed with food and water *ad libitum*.

Collection, Processing and Analysis of EEG and EMG Data

EEG and EMG telemetry signals were acquired at a sampling rate of 250 Hz using a wireless recording device (input voltage: -1.25 to +1.25 mV; Dataquest A.R.T, version 4.1, Data Sciences International) collected through an RPC-2 receiver (Data Sciences International) placed directly beneath the home cage of the recorded animal. The built-in bandwidth of the 240-fold amplified signal was 1-100Hz.

For each rodent, sleep stages had been previously manually scored offline using Neuroscore software (version 2.0.1, Data Sciences International). Epochs of 10 second was staged by the dominant EEG and EMG characteristic of the period (≥50%) into either wakefulness, NREM sleep or REM sleep using Neckelman and Ursins criteria (1993). For the scoring, EEG signals were high pass filtered at 0.5 Hz and low pass filtered at 35 Hz, while EMG signals were high pass filtered at 5 Hz.

Offline fast Fourier transforms (FFT) of unfiltered EEG rawdata were processed in SleepReportNormalize, a custom Matlab-based application designed by Professor Jonathan Wisor, Washington State University, USA (used in Marti et al., 2020). Using this application, EEG power (μV^2/Hz) and EMG (peak-to-peak) was calculated per epoch. Average EEG

power was calculated in the frequency range of 1 to 90 Hz frequency: 1-4 Hz (delta band), 5-7 Hz (low frequency theta), 7-9 Hz (high frequency theta), 80-90 Hz (high gamma band), and 1-90Hz (total power). The theta ratio was calculated by dividing the power in high frequency theta by the power in the low frequency theta. For the preliminary hypothesis driven analysis, power in the 4-8Hz (theta), 11-15Hz (alpha band),15-35 Hz(beta band) and 35-80Hz (low gamma band) was also calculated.

The script allowed sub-staging wakefulness into quiet, intermediary, and active wakefulness. The algorithm used was based on the EMG activity during wakefulness; changes in peak-to-peak amplitude of the entire 24 hours recording. Waking epochs with an amplitude in the lower 33^{th} percentile was staged as quiet wakefulness, whilst the upper 66^{th} percentile was scored as active wakefulness. Epochs in-between was scored as intermediary wakefulness (Grønli et al., 2016).

The script also identified artefacts as epochs which the power 1 Hz bands within the bandwidth 1-20Hz deviated by more than 5 standard deviations from the mean based on the entire baseline recording of that state (Marti et al., 2020). An equivalent artefact removal was performed based on standard deviations above 5 of EMG peak-to-peak within wakefulness. Additionally, epochs where no signal was of EMG or EEG detected was also considered as artefacts. In total across the entire recording of the H2 1.6 ± 0.4% based on the EEG and 0.1±0.1% based on the EMG of all epochs were considered artefacts, with similar amounts for the two rats in H1(**rat B**: 1.3% EEG, 0.1% EMG, **rat B**: 1.4% EEG, 0% EMG). The EEG and EMG of epochs recognized as artefacts using this method was not included in the analysis of the respective signal, however, the scored sleep/wake was kept for calculations of time spent in each state (Neckelmann et al., 1994).

H2 – Exclusion of animals from analysis

Several animals were excluded from analysis. Out of 18 rats, 9 rats were included in analysis. Out of 27 baseline recordings in available for analysis, only 9 were used (Table x).

Table 1. Exclusion criteria

Exclusion of individual rat
Nine rats were excluded from analysis due to using a different transmitter type (F40), which has a limited bandwidth (Grønli et al., 2017). Thus, high gamma power could not be identified in these rats.
One rat was excluded due to technical difficulties with the sleep score file.
One rat was excluded from analysis as ECG artifacts were observed upon inspection of the raw EEG file in the entire recording session across both leads.
One rat was excluded due to the EEG having two bad channels, which was not redeemable by the standard artifact removal procedure.
Exclusion of recording session
Seven recordings were not analyzed because the animal had been recorded on two occasions. In these cases, only the first recording was used to preserve the assumption of independence for paired sample t-tests.

Statistical analyses

Calculations of time in each state, scoring of high gamma wake, and calculation of descriptive statistics were carried out in in Microsoft Excel, while t-tests and graphs were made using Graph pad prism (9.3.0). Values were reported as mean ± SD except where otherwise noted.

In H2, the average power (1-90 Hz) for the entire 24h recording was 71 ± 58 $\mu V^2/Hz$, ranging from 184 to 30 $\mu V^2/Hz$. The average EMG was 238 ± 207 µV, ranging from 52 to 676 µV. Since a parametric paired t-test assumes that the magnitude of differences in the measured variable is constant across subjects, this assumption could be violated by large variations in signal strength. Thus, the EEG and EMG signals were log transformed to

perform ratio paired t-tests. For the high-to-low theta ratio no transformation was performed since it already represented a ratio.

Results

Hypothesis driven analysis

The 24 h sleep-wake pattern.

The rats displayed a typical polyphasic sleep-wake pattern, spending more time awake in the dark phase (Rat A: 72%, Rat B 59%) than the light phase (Rat A: 32%, Rat B 28%). They also exhibited a normal sleep pattern, with most of the sleep dominated by NREM sleep. Percentage NREM sleep of total sleep time was approximately 80% (dark phase: 83% and 85% and light phase: 77% and 77%, Rat A and Rat B respectively).

The hypotheses driven analyses

Wakefulness episodes longer than 5 mins throughout a 24 hrs recording were used for the analyses. Rat A exhibited in total 14 long wakefulness episodes and Rat B 15 long wakefulness episodes.

For each episode of long wakefulness, EEG power in the frequency bands of delta, theta, alpha, beta, low and high gamma were calculated. The analyses identified a distinct recurring pattern of high EEG intensity, stable across multiple epochs in the beta, and low and high gamma frequency bands. A less apparent increase was present in the delta and theta bands, while the EEG power in the alpha band did not show any distinguishable pattern during long periods of wakefulness.

These frequency bands were compared with corresponding time periods of two current ‘quality’ measures of wakefulness based on A) high-to-low frequency theta ratio (Wigren et

al., 2009) and B) EMG (Grønli et al., 2016). Find this illustrated in Figure 3. Figure 3 shows the first long episode of wakefulness after dark onset in the two rats.

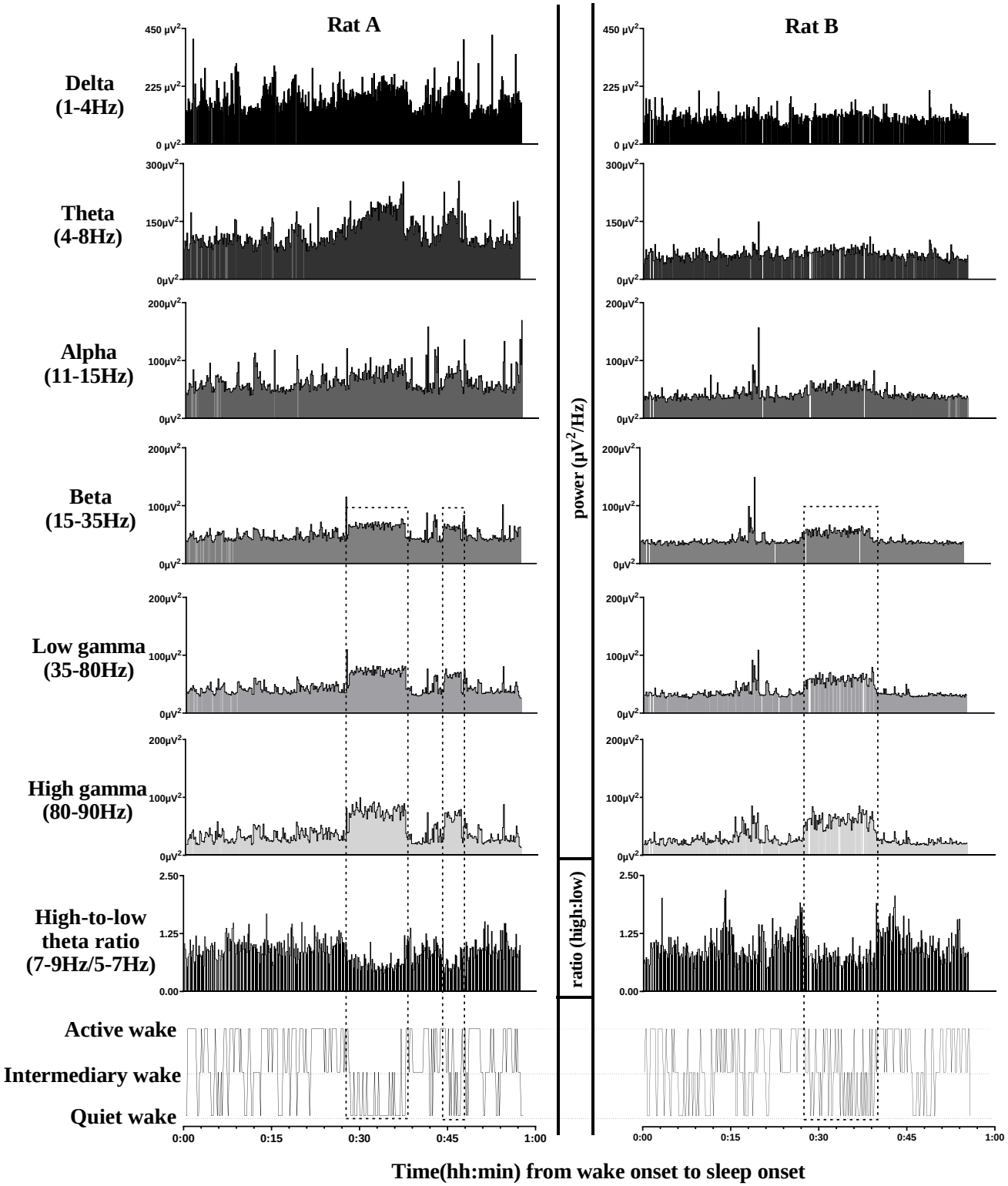

Figure 3. EEG power spectra and measures of wake quality over time during a long period of wakefulness. Averaged EEG power across 10 sec epochs intervals in the delta (1-4Hz), theta (4-8Hz), alpha (11-15Hz), low gamma (35-80Hz) and high gamma (80-90Hz) at frontal-parietal location, in two individual rats. High to low theta ratio and sub-states of wakefulness scored on the basis of EMG are shown in the bottom graphs. The boxed region highlights a pattern of stable increase in power of the high frequency bands concurrent with lower high frequency theta ratio and low activity substates. White bars are identified artefacts by an automated algorithm and removed.

Figure 3 also illustrates a concurrent overlap discovered between the increase in the higher frequency bands and a drop in the high-to-low frequency theta ratio. Furthermore, the EMG based wake-hypnograms revealed an overlap between the quiet wakefulness and the increase in the higher EEG frequencies.

Classification criteria of wakefulness states based on high gamma intensity

To quantify the observation of a stable increase of the higher frequencies as an EEG marker of homeostatic regulation of wakefulness, a classification based on the intensity of high gamma frequency (80-90 Hz) was developed based on its prominent changes during long periods of wakefulness. The classification criteria were developed to distinguish high gamma wakefulness (HGW) and non-high gamma wakefulness (nonHGW) from each other with high specificity to greater intensity to epochs in this band, and with high sensitivity to longer periods of increased power.

The criteria for a binary method of EEG based homeostatic regulated wakefulness were chosen to rely on a percentile score of ≥90%. A 10% reduction from this score included epochs with an approximate value close to the 90^{th} percentile. To increase the sensitivity to longer periods, a criterion was added to include epochs in-between two high intensity epochs. These epochs were confirmed to be of a higher gamma intensity on average compared to other epochs of wakefulness (Rat A: +165%, Rat B: +160%).

To only score longer periods of increased high gamma, a minimum of 4 consecutive epochs were considered HGW. Aiming to compare HGW with other epochs of long wakefulness, nonHGW was scored as all epochs within long wakefulness that did not meet the criteria 1-3. Table 2 lists the scoring criteria.

Table 2. Scoring criteria for HGW and nonHGW

High gamma wake criteria (scored from all wakefulness)
Criterion 1: High gamma power in a scored wake epoch is higher or equal to the 90th percentile (minus 10%) of the entire recording. *Criterion 2:* The epoch is scored as HGW if both the following and preceding epoch was scored as HGW based on criterion 1. *Criterion 3:* Epochs that are scored as HGW based on criteria 1 and 2 were excluded if the consecutive amount of epochs is less than 4.
Non-high gamma wake criterion: All epochs in *long wakefulness* (≥5min) epochs that were excluded by criterion 1-3 are scored as nonHGW
Brief wakefulness: epochs in wakefulness shorter than 5 minutes

HGW – High gamma wake.

Figure 4 illustrates this classification method during the first long wake episode of both rats. Figure 4 also shows overlap between HGW/nonHGW classification and drops in high-to low theta ratio and EMG peak to peak in the animals.

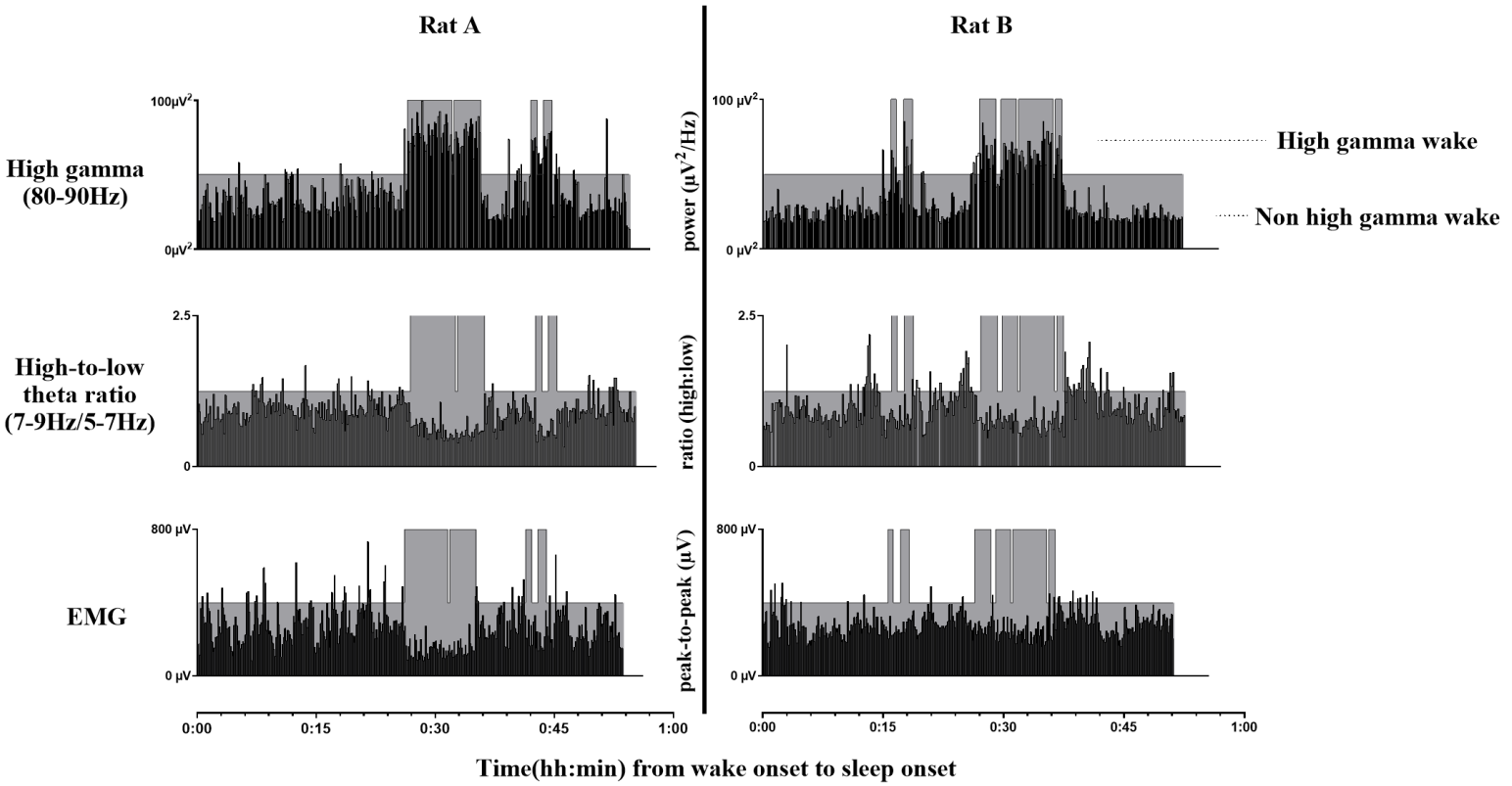

Figure 4. Wakefulness scored by the intensity of high gamma.

The upper graphs illustrate wakefulness scored by the relative power in EEG of the high gamma band (per 10s epoch). The tall and short transparent grey colored bars overlapping each figure represent wake scored as high gamma wake and non-high gamma wake respectively. The middle graphs illustrate the overlap between this classification and the high-to-low theta ratio, while the bottom graph illustrates the overlap with EMG (peak to peak). Each episode was selected as the first long wake episode occurring after dark onset for each rat.

Testing if high gamma intensity may be an EEG marker of homeostatic regulation of wakefulness throughout a 24 hrs recording

Rat A displayed more nonHGW than HGW across undisturbed 24 hrs; a total of 2994 epochs were scored as nonHGW, while 604 were scored as HGW. Similarly, Rat B exhibited 2141 epochs of nonHGW and 499 HGW epochs. Power in the high gamma band was higher in HGW than in nonHGW epochs (Rat A: +133%, Rat B: +166%). No epochs in brief wakefulness met all criteria for high gamma wake. HGW epochs was present in 11 out of the 14 long wake episodes of Rat A, and 7 out of the 15 long wake episodes of Rat B. The long wakefulness episodes where HGW was identified proved a longer duration (Rat A: 50 ± 20

min, Rat B: 50 ± 21 min) compared to wakefulness period without HGW (Rat A: 15 ± 5 min, Rat B: 11 ± 10 min). Figure 5 shows that HGW occurred most frequently in the middle and last part of each long wake bout. Together these findings suggest that the content of high gamma during wakefulness is more present during longer time awake which could reflect a self-regulatory dynamic in wakefulness.

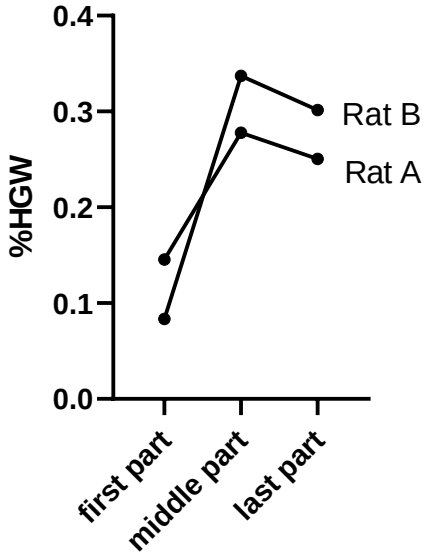

Figure 5: The occurrence of HGW in long wakefulness episodes.

The plot shows the relative occurrence of HGW as compared to nonHGW in each long wake episode (rat A: n=14, rat B: n=15) episode divided into three thirds based on the length of the entire episode. HGW – High gamma wake.

A substantial overlap between HGW and a published measure of quiet wake quality based on high-to-low theta ratio (Wigren et al., 2009) was present. This ratio was lower and less variable in HGW (Rat A: 0.63 ± 0.12, Rat B: 0.76 ± 0.16) than in nonHGW (Rat A: 0.89 ± 0.22, Rat B: 1.04 ± 0.32). The overlap between HGW and quiet wakefulness quantified with low EMG tonus (Grønli et al., 2016) was 36% in Rat A and 30% in Rat B. In nonHGW this overlap was smaller (Rat A: 19%, Rat B: 18%). Relatedly, the EMG per epoch was lower on average in HGW than nonHGW irrespective of percentile distribution (Rat A: 26% lower, Rat

B: 13% lower). Comparison of HGW with literature classification of quiet wakefulness with favored that HGW was a relatively low arousal, quiescent state of the long wakefulness episodes.

If HGW reflects compensatory mechanisms allowing animals to restore metabolic processes during the waking state, HGW would be associated with changes in sleep pressure that builds up during time in wakefulness. Amount of SWA in quiet wakefulness is consistently found to be a marker of sleep pressure and found to be correlated with restorative metabolic processes in the cortex (Grønli et al., 2017; Grønli et al., 2016; Marti et al., 2020; Wisor et al., 2013). Indeed, SWA was found to be higher in HGW than in nonHGW, for both rats; +19% in Rat A and + 9% in Rat B.

Notably, Rat A spent more time in long episodes of wakefulness (+2hrs 40min), exhibited the lowest high-to-low theta ratio during HGW (-0.13) and nonHGW(-0.15) and the highest relative difference of EMG (+50%) and SWA (+53%) of the two rats. If HGW is found to be a marker of homeostatically regulated wakefulness, these differences could be used to assess the intensity of regulation. Hence, a possible method-model association was identified.

Although far from conclusive, the preliminary findings endorsed that the intensity of high gamma frequencies may be used as a homeostatic marker of wake regulation. If this hypothesis is to be verified, the following should be true; 1) the hypothesis holds in a larger population, 2) the hypothesis holds across different cortical EEG deviations and 3) the hypothesis holds during both light phase and dark phase.

If the above tests verified in an undisturbed population, experimental conditions challenging time in wakefulness can be tested (i.e. simulation of shift work, changes in L:D schedule, different stress exposures).

Testing the hypothesis that high intensity gamma is an EEG marker of homeostatic regulated wakefulness

The descriptive analyses of HGW/nonHGW were extended to nine rats and across two EEG deviations; frontal-parietal (FP) and frontal-frontal (FF). All rats were single housed and undisturbed. They exhibited a normal pattern of sleep and wakefulness throughout a 24 hrs recording, where the rats spent more time awake during the dark phase (67 ± 8%) than during the light phase (25 ± 7%). Their sleep was dominated by NREM sleep (**D:** 85 ± 3 %, **L:** 78 ± 3 %).

The rats displayed more nonHGW than HGW across undisturbed 24hrs; On average, 2635 ± 324 epochs were scored as nonHGW, while 489 ± 68 epochs were scored as HGW. Power in the high gamma band was 118 ± 53% higher in HGW compared to nonHGW epochs. In accordance with preliminary findings, the peaks in high gamma power were associated with long wake episodes. No epochs in brief wakefulness episodes met all criteria for high gamma wake. HGW was identified in 88 of the total 136 long wakefulness episodes. For the calculation of episode duration, four episodes were excluded due to ending or beginning outside the 24-hour recording. The episodes where HGW was identified proved a longer duration (46 ± 32 min, n=87) than episodes without HGW (12 ± 9 min, n=46). In contrast to the preliminary findings, the occurrence of HGW now dominated the first and middle part of the episodes (Figure 6).

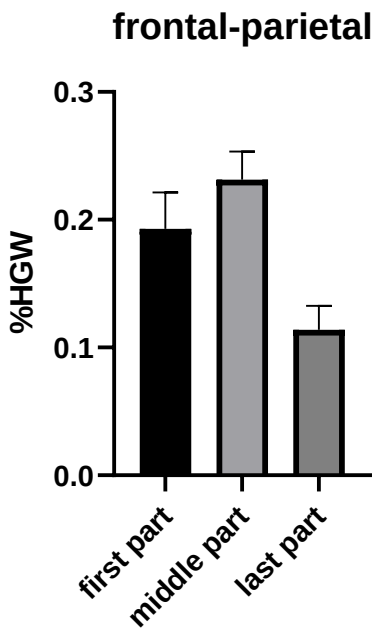

Figure 6: The occurrence of frontal-parietal HGW in long wakefulness.

The plot shows the relative occurrence of HGW as compared to nonHGW across all long wake episodes (n=136), of nine rats. Episodes are divided into three thirds based on the total length of each episode. Data are shown as mean ± SEM. HGW – High gamma wake.

To test the hypothesis that HGW identifies quiet wakefulness, the high to low theta ratio, EMG based score and EMG per epoch was compared with nonHGW. Consistent with preliminary findings, the ratio of high to low theta frequency was significantly lower during HGW than in nonHGW (*.24* ± .14, t(8)= 5.03, p=.001). However, the overlap between HGW and quiet wakefulness quantified by low EMG tone distribution was lower in HGW (19 ± 15%) than in nonHGW (25 ± 5%). No significant difference in EMG per epoch was found between HGW and nonHGW (.99 ± 0.03, $t(8) = 0.25$, $p = .806$). As in the preliminary analysis, the homeostatic marker SWA tended to be higher in HGW than in nonHGW (0.94 ± 0.04, $t(8) = 1.90$, $p = .094$).

Comparisons of EEG locations for classification of HGW and nonHGW

All analyses done so far were performed with EEG information from the frontal-parietal deviation. Now, all epochs scored as wakefulness were classified into HGW and nonHGW based on EEG information from the frontal-frontal cortical area. An average of 2636 ± 328 epochs in FF was scored as nonHGW, and the amount of HGW was 487 ± 71. There was a substantial overlap between HGW in scored by FF EEG and HGW scored by FP EEG (75.2 ± 17.2%). Power in the high gamma band was 97 ± 51% higher in HGW compared to nonHGW epochs. Again, no epochs of brief wakefulness met all criteria for HGW. HGW was identified in 90 long wakefulness episodes. Episodes where HGW was identified proved a longer duration (45 ± 32 min, n=89) than episodes without HGW (13 ± 11 min, n=43). Figure 7 shows that the distribution of HGW was skewed towards the first and middle parts of the wakefulness episode.

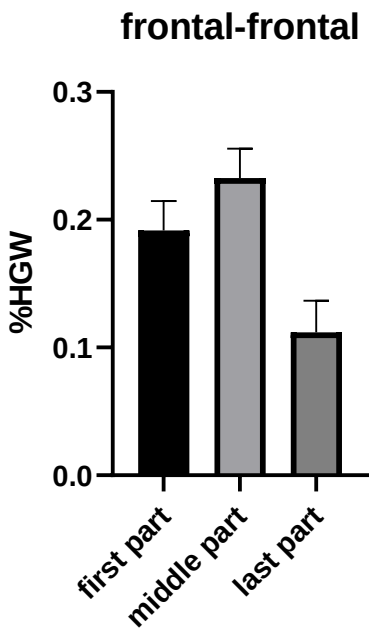

Figure 7: The occurrence of frontal-frontal HGW in long wakefulness.

The plot shows the relative occurrence of HGW as compared to nonHGW across all long wake episodes (n=136), of nine rats. Episodes are divided into three thirds based on the total length of each episode. Data are shown as mean ± SEM. HGW – High gamma wake.

The same comparisons between HGW and nonHGW as in the FP lead were followed up by equivalent comparisons in the FF lead. The high-to low theta ratio was no longer significantly lower in HGW compared to nonHGW (0.14 ± 0.23, t(8)=1.91, p=.092). The other analyses paralleled results from FP scoring: a lower overlap between EMG quantified quiet wakefulness and HGW (19 ± 14%) was found than in nonHGW (25 ± 5%). Like in the FP, no significant difference in EMG between HGW and nonHGW was found (0.98 ± 0.04, t(8) = .84, p = .425). Again, the FF SWA tended to be higher in HGW compared to nonHGW (0.87 ± 0.09, $t(8)=2.06$, $p=.074$).

HGW and nonHGW in dark phase and light phase

All analyses done so far were based on the entire 24h recording. Now, the dynamics of FF/FP HGW and nonHGW were compared in the light and dark phase.

Figure 8 illustrates that both nonHGW and HGW (scored from FP) were more present during the dark phase than light phase. A drop in time spent in HGW was evident 2 to 4 hrs into the dark phase (ZT14-16).

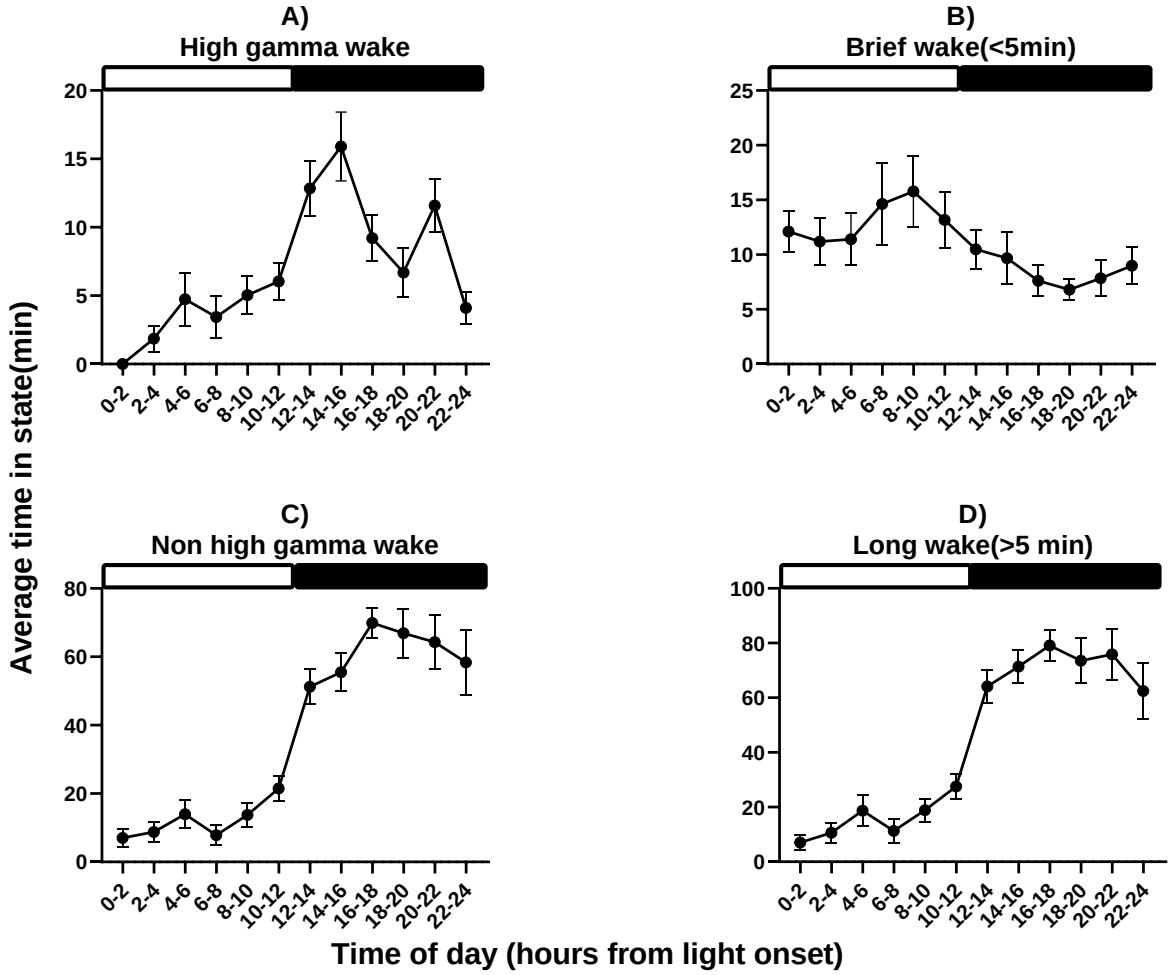

Figure 8. The average time spent in each state in a full day in undisturbed conditions in home cage for nine rats. The white and black bar indicate light condition. A) The amount of time spent in high gamma wake was greater during the dark phase with peaks at ZT14-16 and ZT20-22. B) On average the rats spent more time in brief wake episodes during the light phase than during the dark phase. C) Non high gamma wake is wake that is in long wake episodes (shown in figure D) which are not scored as HGW. Data are shown as mean ± SEM. Note that different scales are used for each graph.

To investigate whether there were systematic differences in the distribution of HGW and nonHGW, the ratio of these states was compared between light and dark phase. Figure 9 illustrates that the ratio between HGW and nonHGW was highest during the light phase, scored from both FP and FF deviations.

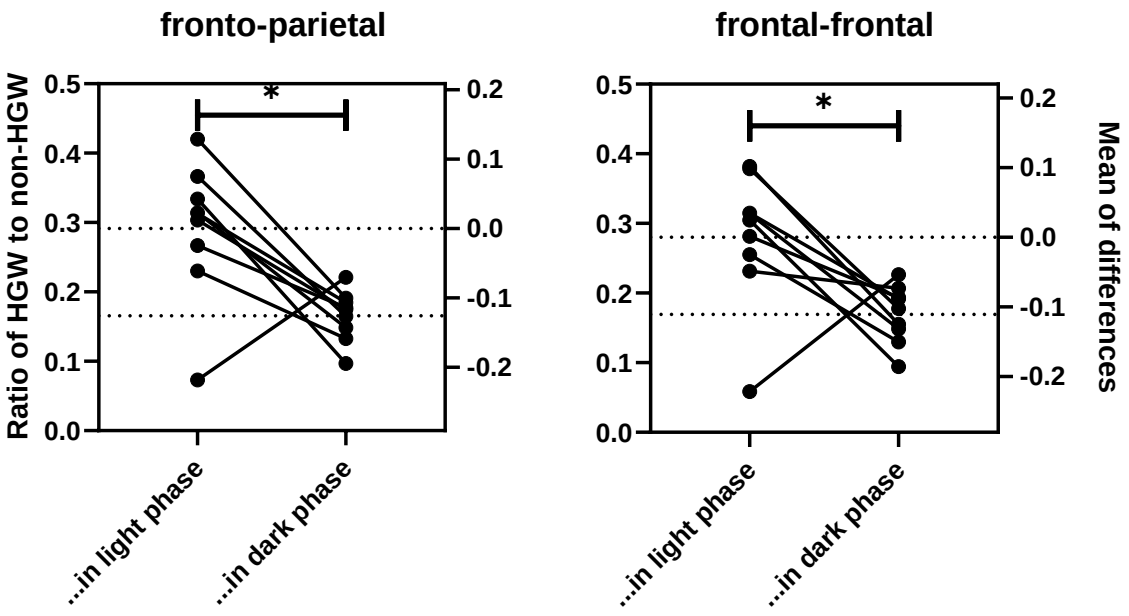

Figure 9. Estimation plots of paired t-tests comparing the ratio of HGW to nonHGW in light versus dark phase. Each point represents the ratio for one individual rat in the corresponding light condition. The ratio was significantly lower in the dark phase for both frontal-parietal (left plot, $p = .012$) and frontal-frontal (right plot, $p = .027$) EEG leads. HGW – High gamma wake.

Finally, to investigate whether the differences in light and dark phase impacted the results, HGW and nonHGW were compared separately in each phase. The direction and significance of results were similar to the analysis based on the entire recording for high-to-low theta ratio (Table 3), EMG and SWA (Table 4).

Table 3. High-to-low theta ratio in the light and dark phase

		Mean difference (nonHGW - HGW)	*SD*	*t*	*p-value*
	Light phase				
FP		0.17	0.13	4.09	.004
FF		0.12	0.16	2.26	.054
	Dark phase				
FP		0.25	0.14	5.48	<.001
FF		0.15	0.24	2.26	.103

n=9, comparisons were made using paired t-test. FF - frontal-frontal, FP - frontal-parietal, HGW - high gamma wake

Table 4: Comparing EMG and SWA in the light and dark phase

	Measure	*Geometric mean of ratios* (nonHGW/HGW)	*SD*	*t*	*p-value*
	Light phase				
FP	EMG	0.90	0.08	1.76	.116
	SWA	0.95	0.05	1.38	.021
FF	EMG	**0.88**	0.09	1.91	.093
	SWA	0.90	0.10	1.36	.210
	Dark phase				
FP	EMG	1.01	0.03	0.33	.754
	SWA	0.93	0.05	2.15	.064

FF	EMG	0.99	0.03	0.34	.741
	SWA	0.85	0.09	2.30	.051

n=9, comparisons were made using paired t-test. FF - frontal-frontal, FP - frontal-parietal, HGW - high gamma wake

Discussion

Active wakefulness is crucial for motivated behaviours that increase reproductive success. The adaptive value of active wakefulness is limited. As we stay actively awake for long periods, performance and health may become impaired. Thus, active wakefulness reduces our adaptive wakefulness capacity. The effects of quiet wakefulness in relation to adaptive wakefulness capacity is poorly understood. Several studies suggest that sleep-like EEG events may occur during wakefulness. In addition, restorative properties associated with sleep homeostasis have also been observed during wakefulness. Since the restorative events may be linked to performance impairment, and depend on reduction in signaling activity, I suggested that quiet wakefulness and sleep were ideal states to consolidate these processes globally. The restorative events were linked to reduction in homeostatic pressure of signaling molecules such as adenosine. If these hypotheses hold true, it may be expected that quiet wakefulness is homeostatically regulated. It may also be expected that quiet wakefulness may increase adaptive wakefulness capacity, either through increasing the length of wakefulness or through enabling more adaptive use of active wakefulness. These are the core predictions of the adaptive wakefulness capacity model.

To investigate these predictions, a hypothesis driven approach was appropriate. I attempted to identify EEG markers of homeostatically regulated quiet wakefulness associated with longer, undisturbed, wake episodes in rats. In the preliminary analysis of two rats, temporally close epochs with intense high gamma (HGW) were identified as a possible

marker of homeostatically regulated quiet wakefulness. These occurred concurrently with a low high-to-low theta ratio and low EMG, markers of quiet wakefulness. In addition, they occurred at later stages of longer wakefulness episodes and displayed higher SWA than other epochs in long wakefulness.

Amongst these hypothetical relationships, the association between HGW and 1) SWA, 2) lower EMG and 3) its occurrence in latter stages were falsified when analysis was extended to nine rats. Support was found for the association between HGW and longer wakefulness episodes, as well as a lower high-to-low theta ratio.

These findings may be of relevance for future investigations into the homeostatic regulation of quiet wakefulness. The results suggest that high gamma is in some way associated with longer wakefulness episodes. Support for this conclusion was found in all rats, in both frontal-frontal and frontal-parietal EEG leads. This parallels findings in mice hippocampal EEG, suggesting that high gamma (>80Hz) is significantly higher in long wake episodes (Perez-Atencio et al., 2018). Interestingly, Perez-Atencio and colleagues (2018) reported that the power in hippocampal 7-10Hz theta was significantly higher in the long wake episodes of mice, while theta peaked at 6 Hz in brief wake episodes. The current findings suggest that increases in high gamma of long wake episodes is dominated by lower frequency theta in the frontal-parietal EEG, and the same trend, although not significant, was found for frontal-frontal EEG. Thus, the association between high gamma and long wakefulness might be related to different parts of the episodes than that of theta.

The failure to find a relationship between HGW and decreased EMG relative to the rest of the long wake episode warrants discussion. Three different and plausible explanations can be offered for this observation.

First, it could be argued that HGW is a marker of active wakefulness, rather than quiet wakefulness. Power in the high gamma has been reported to increase with sensory stimulation in sleep deprivation sessions of mice (Grønli et al., 2016). This explanation is also compatible with the association with HGW and longer wakefulness, as it could be argued that low basal levels of arousal limit the length of wakefulness in laboratory settings. It is also worth noting that HGW was a larger part of the long wakefulness episodes during the rest (light) phase, than the active (dark) phase. If HGW is associated with higher arousal wakefulness, the finding may suggest that the amount of high gamma required to sustain an episode may be higher during the light phase due to a lower baseline level of arousal.

A second possibility for explaining the results is that HGW, as hypothesized, identifies homeostatically regulated quiet wakefulness in long wake episodes. This view is supported by the concurrent lowered ratio of high frequency theta that was observed in the fronto-parietal EEG 11 out of 11 rats. As discussed, a lower peak frequency of theta is associated with quiet wakefulness and lower arousal in the literature (Neckelmann & Ursin, 1993; Vyazovskiy & Tobler, 2005; Wigren et al., 2009; Young & McNaughton, 2008).

There is also an intriguing possibility that the high gamma intensity is related to frequent occurrence of sharp wave ripple activity. The observation that the high gamma band displayed high intensity during low-EMG epochs (for Rat A and Rat B) supports this view, as sharp wave ripples are reported to be the only high amplitude event in the alert but immobile animal (Buzsáki, 2015). Sharp wave ripples are also associated with gamma oscillations (30-120Hz) in the hippocampus (Buzsáki, 2015). The fact that there was no significant relationship between EMG and HGW is not necessarily a contradiction, as sharp wave ripples occur frequently during high EMG automatic behaviours, including grooming and eating (Buzsáki, 2015).

If it can be shown that the high gamma intensity is strongly correlated with quiescent behaviours and sharp wave ripples, the finding that these occur in longer wakefulness episode is of high relevance to the adaptive wakefulness capacity model. Sharp wave ripples occur during offline states of the brain and has been linked to wakeful waste clearing activity. Optogenetic induction of gamma waves at 40Hz during ripple activity in the hippocampus has been shown to upregulate microglial volume, reduce production of Aβ and effectively reduce Aβ load in awake mice (Iaccarino et al., 2016). In addition, hippocampal sharp waves have recently been linked to homeostatic regulation of external glucose metabolism (Tingley et al., 2021), possibly supporting the suggestion that reduction in aerobic glycolysis is necessarily a part of reduction in waste and regeneration of metabolic energy. However, the lack of behavioural and hippocampal local field potential recording means that the relationship between HGW and SPW-Rs remains speculative at best.

A third plausible explanation of the findings is a combination of the two listed above. It is possible that stable peaks in high gamma reflects a less homogeneous state than assumed in my analysis. This is supported for gamma in the lower ranges (30.5-58.0 Hz), which have been shown to correlate with a wide range behaviours, such as eating, grooming, moving and alert immobility (Maloney et al., 1997). If the high gamma band is similar in nature to the low gamma band, then it is possible that the observations reported here only suggest that the animal engages in behaviour in long wakefulness episodes, while not engaging in behaviour in brief ones.

Some further limitations to interpretation of results warrant discussion. As discussed, laboratory settings demonstrably impact the wakefulness regulation of the animal. The arousal promoting factors is virtually absent compared to wild conditions. The animal will not have predatory encounters, nor mating opportunities. It does not need to forage its food, explore novel objects, and may eat and drink as it pleases. It is also habituated to its home

environment, so unless it is stressed, it is likely that active wakefulness is reduced compared to wild conditions. Considering these limitations, it is also possible that quiet wakefulness occurs as a default state in the absence of arousal promotion. The rat might simply lack something to do. In addition, it is suggested that the likelihood of predatory encounters and opportunities, and capacity to respond efficiently determines whether the animal should sleep or rest since. Thus, in laboratory environments the rats may choose to sleep instead of restitution in quiet wakefulness in response to homeostatic pressure. To assess the impact of these manipulations, results may be compared with recordings in the wild, which currently is limited by the need to perform telemetric recording to identify vigilance states. Another possibility is to create more naturalistic experimental settings, but the cost, practicality, and difficulties in interpreting the relative influence of variables under recording in these conditions must also be appreciated.

I used EEG recording to identify the outlined homeostatic properties of quiet wakefulness. This is consistent with the methods used in sleep homeostasis research. EEG can be noisy, impacted by movement artifacts, is sensitive to recording conditions and has poor spatial resolution (Jackson & Bolger, 2014). The high-to-low theta ratio measure used here also differs in some ways from the publication it was adapted from. Wigren and colleagues (2009) recorded fronto parietal EEG with slightly different lambda coordinates, at the dura mater rather than on the skull, and used bipolar screw EEG. The impact of these differences on the recorded EEG remains uncertain. In addition, the interpretation that certain EEG frequency bands may identify metabolic changes in the brain was supported by correlational evidence. Ideally, a study attempting to falsify homeostatic regulation of quiet wakefulness should assess metabolic changes in wakefulness regulating regions directly.

The scoring method used for identifying stable high gamma intensity had biases that may have impacted the results. In part, the scoring criteria was based on percentile of all

wakefulness, which was done because the high gamma intensity was relative. This biases the scoring to the total length of wakefulness. To compensate for the bias, a 10% reduction was added to the scoring, thus if a rat had more epochs close in value to the 10^{th} percentile, more wakefulness would be scored as high gamma. Also, the method ensures that at least 10 percent of all wakefulness is eligible for being scored as high gamma. Thus, if a rat had less periods of high intensity gamma, the scoring method would overestimate the amount. For this reason, a conservative percentile and percent was chosen. Arbitrary percentile-based scoring is common in published literature, but typically the authors report behavioural confirmation of the scoring method. This is needed to conclude that scoring was successful in identifying a state.

Ideally, a state of quiet wakefulness during long wakefulness episodes is identified using a method that is independent on the total amount of wakefulness of recording. A promising approach could be to record local field potentials in the hippocampus for sharp wave ripples. As these are events and not relative measures, the frequency of these events during wakefulness could be used to identify the state.

Despite the limitations and ambiguity of results, the current study reveals two important guidelines for future operationalizations of quiet wakefulness. The first is confirmation that brief wakefulness episodes have different electrophysiological characteristics than long wakefulness in rats. This should be considered when comparing rodent wakefulness with human wakefulness, since human wakefulness is often consolidated in long periods. The second is that in long wakefulness episodes EMG may be relatively high when the high-to low theta ratio is identified as low. This means that either both measures are insufficient, or one of them may be insufficient to score quiet wakefulness.

Future directions of the model

The value of any model comes from the accuracy and usefulness of its predictions. While the validity of the core hypothesis remains uncertain, there are several interesting secondary hypotheses that can be predicted from the perspective of the model. The model offers new perspectives on three phenomena with large potential utility: the post lunch dip, Alzheimer disease and sleep pressure.

The post lunch dip

A dip in performance-levels and alertness (and increased sleepiness) after noon called the "post lunch dip" has been documented in humans (Carskadon & Dement, 1992). This has been explained by a mismatch between sleep pressure and circadian rhythm. As wakefulness progresses into noon-afternoon hours, sleep pressure increases. This is counteracted by a circadian modulated increase in alertness labelled the "wake maintenance zone". In a constant routine study, the peak of the wake maintenance zone occurred around 19-23:00 in adults (Zeeuw et al., 2018). Thus, the wake maintenance zone peak occurs "too late" to counteract the increased sleep pressure during the post-lunch hours (Bes et al., 2009)

The adaptive wakefulness capacity model offers a unique perspective on this phenomenon. As the post lunch dip occurs after a prolonged period of wakefulness, it is possible that waste products have accumulated, and metabolic energy have been expended in signalling activity. Accordingly, the pressure to quietly rest or sleep may be high. If the arousal levels are sufficiently low, it may be expected that the post lunch dip offers restitution, increasing brain metabolic energy and decreasing metabolic waste. This would increase the adaptive wakefulness capacity.

Falsifying this hypothesis is difficult, as we cannot compare the effects of active wakefulness versus quiet wakefulness during the post lunch dip and conclude whether quiet

wakefulness was restorative. Any effects of this manipulation could be attributed to increased use-limiting effects of additional active wakefulness. Instead, a more promising prospect is to compare metabolite- or homeostatic marker concentration in wake promoting regions in subjects before and after the post-lunch dip.

If the post lunch dip is a restorative period, high arousal could be more harmful within than outside it, because the post lunch dip period comes before the wakefulness maintenance zone. During the wakefulness maintenance zone alertness levels are increased, and sleep-propensity is low (Zeeuw et al., 2018). Thus, after the post lunch dip the restorative homeostasis may be countered by increased arousal. In this regard, confirming the hypothesis would offer a simple and practical perspective to improve performance and health outcomes; avoid active wakefulness during the post lunch dip.

Alzheimer disease

The link between Alzheimer and sleep-deprivation has been related to accumulation in Aβ (Wu et al., 2019). Consequently, there may be a link between Alzheimer's and impaired quiet wakefulness. If both sleep and quiet wakefulness are involved in clearance of Aβ, there might be co-morbidity in risk of Alzheimer when both sleep and quiet wakefulness is impaired. Resting state EEG of human Alzheimer patients consistently reveal lower alpha activity in quiet wakefulness compared to age matched control which has been interpreted as heightened arousal (Babiloni et al., 2017). They also display higher theta activity (Jelic et al., 2000), a marker of homeostatic sleep pressure in human wakefulness (Finelli et al., 2000). From the perspective of the model, it is possible that the high arousal during quiet wakefulness indicates inadequate response to the homeostatic pressure. Due to the high prevalence and devastating impact of Alzheimer disease, further investigations into the

relationship between quiet wakefulness and Aβ clearance should be an objective of future research.

Sleep pressure

The model suggests that quiet wakefulness may be effective in reducing the homeostatic sleep pressure, due to reduction in the metabolic products that generates it. Thus, a counter-intuitive prediction of the model is that if wakefulness is extended with quiet rather than active wakefulness, sleep-pressure may be reduced rather than increased. A comparison of sleep deprivation in mice found that SWA in NREM after sleep deprivation by automatic behaviours (simple touch screen task) was much lower than after inducing exploratory or voluntary behavior (wheel running, presentation of objects; Milinski et al., 2021). As this finding could be attributed to relative increased demand of active wakefulness, this cannot be considered evidence of the hypothesis that quiet wakefulness reduces sleep pressure. Indeed, falsification of this hypothesis is difficult.

Several complications must be addressed. The most pressing issue is that the metabolic demand may not be equal across wakefulness, as suggested by the synaptic homeostasis model (Vyazovskiy et al., 2008). If this hypothesis is correct, the late phase quiet wakefulness will be less effective at restorative processes than the early phase quiet wakefulness. Thus, a lower arousal quiet wakefulness may be needed to reduce sleep pressure during the late stages than early stages of the day. Another concern is that it is difficult to imagine a way to reliably induce extended wakefulness in a way that ensures it is quiet. For instance, the operant touch screen task described by Milinski and colleagues (2021), may plausibly induce either a less aroused active wakefulness, or a state of quiet wakefulness. Distinguishing between the two is crucial to test the hypothesis. A final concern is that at the end of a normal day of wakefulness, the circadian modulation of arousal decelerates. Thus, if

subjects are compared with baseline levels (at normal sleep times) in terms of slow-wave activity to measure the homeostatic response, there may be confounding effects of circadian regulation if the subjects are kept awake for longer. Instead, an ideal experimental method could involve a quiet wake-inducing sleep deprivation technique concurrently with markers of homeostatic sleep drive. An example could be to measure extracellular concentration of adenosine in a wakefulness promoting region while the subjects are quietly awake.

A practical implication of this hypothesis is that avoiding prolonged quiet wakefulness before going to bed ensures that the negative feedback mechanism decreasing arousal remains intact. If the negative feedback is reduced by restoration, any arousal promoting factors are likely to be more effective. Thus, knowledge of whether quiet wake is restorative may be useful to improve night-time sleep. It must be stressed that the aim of this model is not to suggest that quiet wakefulness may replace sleep. Rather, this model suggests that quiet wakefulness is less efficient at restoration than sleep since sleep allow greater reduction in metabolic demand.

Throughout this thesis, I have argued that adaptive wakefulness capacity is limited by the performance and health outcomes of being in active wakefulness. Evidence from sleep homeostasis research suggest that this capacity is reduced by high metabolic demand and restored by sleep. The role of quiet wakefulness has largely been ignored in sleep homeostasis research, but the limited research that exist suggest that the capacity may also be increased during this state. In addition, arousal inhibiting mechanisms involved in sleep homeostasis also act on pathways involved in regulation of quiet wakefulness. Results from the hypothesis driven analysis suggest that future operationalization of quiet wakefulness should distinguish between long and short wakefulness episodes, include behavioural data and record hippocampal local field potential. In conclusion, I have not been able to demonstrate that

quiet wakefulness is homeostatically regulated to increase adaptive wakefulness capacity. I suggest that this question is of considerable interest for future research.

References

Abel, T., Havekes, R., Saletin, J. M., & Walker, M. P. (2013). Sleep, plasticity and memory from molecules to whole-brain networks. *Current biology, 23*(17), R774-R788.

Alexandre, C., Andermann, M. L., & Scammell, T. E. (2013, 2013/10/01/). Control of arousal by the orexin neurons. *Current Opinion in Neurobiology, 23*(5), 752-759. https://doi.org/https://doi.org/10.1016/j.conb.2013.04.008

Aschoff, J. (1965, Jun 11). CIRCADIAN RHYTHMS IN MAN. *Science, 148*(3676), 1427-1432. https://doi.org/10.1126/science.148.3676.1427

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *Journal of Cerebral Blood Flow & Metabolism, 21*(10), 1133-1145.

Babiloni, C., Del Percio, C., Lizio, R., Noce, G., Cordone, S., Lopez, S., Soricelli, A., Ferri, R., Pascarelli, M. T., Nobili, F., Arnaldi, D., Aarsland, D., Orzi, F., Buttinelli, C., Giubilei, F., Onofrj, M., Stocchi, F., Stirpe, P., Fuhr, P., Gschwandtner, U., Ransmayr, G., Caravias, G., Garn, H., Sorpresi, F., Pievani, M., Frisoni, G. B., D'Antonio, F., De Lena, C., Güntekin, B., Hanoğlu, L., Başar, E., Yener, G., Emek-Savaş, D. D., Triggiani, A. I., Franciotti, R., De Pandis, M. F., & Bonanni, L. (2017, 2017/07/01/). Abnormalities of cortical neural synchronization mechanisms in patients with dementia due to Alzheimer's and Lewy body diseases: an EEG study. *Neurobiology of Aging, 55*, 143-158. https://doi.org/https://doi.org/10.1016/j.neurobiolaging.2017.03.030

Benington, J. H., & Heller, H. C. (1995, Mar). Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol, 45*(4), 347-360. https://doi.org/10.1016/0301-0082(94)00057-o

Berger, R. J., & Phillips, N. H. (1995, Jul-Aug). Energy conservation and sleep. *Behav Brain Res, 69*(1-2), 65-73. https://doi.org/10.1016/0166-4328(95)00002-b

Bes, F., Jobert, M., & Schulz, H. (2009). Modeling napping, post-lunch dip, and other variations in human sleep propensity. *Sleep, 32*(3), 392-398.

Bloch, G., Herzog, E. D., Levine, J. D., & Schwartz, W. J. (2013). Socially synchronized circadian oscillators. *Proceedings of the royal society B: biological sciences, 280*(1765), 20130035.

Blouin, A. M., Fried, I., Wilson, C. L., Staba, R. J., Behnke, E. J., Lam, H. A., Maidment, N. T., Karlsson, K. Æ., Lapierre, J. L., & Siegel, J. M. (2013, 2013/03/05). Human hypocretin and melanin-concentrating hormone levels are linked to emotion and social interaction. *Nature Communications, 4*(1), 1547. https://doi.org/10.1038/ncomms2461

Bojarskaite, L., Bjørnstad, D. M., Pettersen, K. H., Cunen, C., Hermansen, G. H., Åbjørsbråten, K. S., Chambers, A. R., Sprengel, R., Vervaeke, K., & Tang, W. (2020). Astrocytic Ca 2+ signaling is reduced during sleep and is involved in the regulation of slow wave sleep. *Nature Communications, 11*(1), 1-16.

Borbély, A. A. (1977). Sleep in the rat during food deprivation and subsequent restitution of food. *Brain Research, 124*(3), 457-471.

Borbély, A. A. (1982). A two process model of sleep regulation. *Hum neurobiol, 1*(3), 195-204.

Borbely, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016, Apr). The two-process model of sleep regulation: a reappraisal. *J Sleep Res, 25*(2), 131-143. https://doi.org/10.1111/jsr.12371

Borbély, A. A., & Neuhaus, H. U. (1978, 1978/03/01). Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. *Journal of comparative physiology, 128*(1), 37-46. https://doi.org/10.1007/BF00668371

Brown, R. E., Basheer, R., McKenna, J. T., Strecker, R. E., & McCarley, R. W. (2012). Control of sleep and wakefulness. *Physiological reviews*.

Buhr, E. D., Yoo, S.-H., & Takahashi, J. S. (2010). Temperature as a universal resetting cue for mammalian circadian oscillators. *Science, 330*(6002), 379-385.

Burn, C. C. (2008, 2008/07/01/). What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Applied Animal Behaviour Science, 112*(1), 1-32. https://doi.org/https://doi.org/10.1016/j.applanim.2008.02.007

Buzsáki, G. (2015). Hippocampal sharp wave-ripple: A cognitive biomarker for episodic memory and planning. *Hippocampus, 25*(10), 1073-1188.

Buzsáki, G., Logothetis, N., & Singer, W. (2013, 2013/10/30/). Scaling Brain Size, Keeping Timing: Evolutionary Preservation of Brain Rhythms. *Neuron, 80*(3), 751-764. https://doi.org/https://doi.org/10.1016/j.neuron.2013.10.002

Cain, S. W., Silva, E. J., Chang, A. M., Ronda, J. M., & Duffy, J. F. (2011, Jun). One night of sleep deprivation affects reaction time, but not interference or facilitation in a Stroop task. *Brain Cogn, 76*(1), 37-42. https://doi.org/10.1016/j.bandc.2011.03.005

Carr, M. F., Karlsson, M. P., & Frank, L. M. (2012). Transient slow gamma synchrony underlies hippocampal memory replay. *Neuron, 75*(4), 700-713.

Carskadon, M. A., & Dement, W. C. (1992). Multiple sleep latency tests during the constant routine. *Sleep, 15*(5), 396-399.

Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., Richardson, J. A., Williams, S. C., Xiong, Y., & Kisanuki, Y. (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell, 98*(4), 437-451.

Cirelli, C., & Tononi, G. (2008). Is sleep essential? *PLoS biology, 6*(8), e216.

Danguir, J., & Nicolaidis, S. (1979, 1979/04/01/). Dependence of sleep on nutrients' availability. *Physiology & Behavior, 22*(4), 735-740. https://doi.org/https://doi.org/10.1016/0031-9384(79)90240-3

De Lecea, L., Kilduff, T., Peyron, C., Gao, X.-B., Foye, P., Danielson, P., Fukuhara, C., Battenberg, E., Gautvik, V., & Bartlett, F. n. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proceedings of the National Academy of Sciences, 95*(1), 322-327.

Del Bo, A., Ledoux, J. E., Tucker, L. W., Harshfield, G. A., & Reis, D. J. (1982). Arterial pressure and heart rate changes during natural sleep in rat. *Physiology & Behavior, 28*(3), 425-429.

Dewasmes, G., Duchamp, C., & Minaire, Y. (1989). Sleep changes in fasting rats. *Physiology & Behavior, 46*(2), 179-184. https://doi.org/10.1016/0031-9384(89)90252-7

Dienel, G. A. (2019). Brain glucose metabolism: integration of energetics with function. *Physiological reviews, 99*(1), 949-1045.

Dijk, D., Brunner, D. P., & Borbely, A. A. (1990). Time course of EEG power density during long sleep in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 258*(3), R650-R661.

Dijk, D. J., Brunner, D. P., Beersma, D. G., & Borbely, A. A. (1990, Oct). Electroencephalogram power density and slow wave sleep as a function of prior waking and circadian phase. *Sleep, 13*(5), 430-440. https://doi.org/10.1093/sleep/13.5.430

Dinges, D. F. (1995). An overview of sleepiness and accidents. *Journal of sleep research, 4*, 4-14.

DiNuzzo, M., & Nedergaard, M. (2017). Brain energetics during the sleep–wake cycle. *Current Opinion in Neurobiology, 47*, 65-72. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5732842/pdf/nihms908215.pdf

Doran, S. M., Van Dongen, H. P., & Dinges, D. F. (2001). Sustained attention performance during sleep deprivation: evidence of state instability. *Archives italiennes de biologie, 139*(3), 253-267.

Dubowy, C., & Sehgal, A. (2017, Apr). Circadian Rhythms and Sleep in Drosophila melanogaster. *Genetics, 205*(4), 1373-1397. https://doi.org/10.1534/genetics.115.185157

Daan, S., Beersma, D. G., & Borbely, A. A. (1984, Feb). Timing of human sleep: recovery process gated by a circadian pacemaker. *Am J Physiol, 246*(2 Pt 2), R161-183. https://doi.org/10.1152/ajpregu.1984.246.2.R161

Eban-Rothschild, A., Appelbaum, L., & de Lecea, L. (2018). Neuronal mechanisms for sleep/wake regulation and modulatory drive. *Neuropsychopharmacology, 43*(5), 937-952.

Elbaz, I., Foulkes, N., Gothilf, Y., & Appelbaum, L. (2013, 2013-February-01). Circadian clocks, rhythmic synaptic plasticity and the sleep-wake cycle in zebrafish [Mini Review]. *Frontiers in Neural Circuits, 7*. https://doi.org/10.3389/fncir.2013.00009

Finelli, L., Baumann, H., Borbély, A., & Achermann, P. (2000). Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. *Neuroscience, 101*(3), 523-529.

Foster, R., & Kreitzman, L. (2017). *Circadian rhythms: a very short introduction*. Oxford University Press.

Franzen, P. L., Siegle, G. J., & Buysse, D. J. (2008). Relationships between affect, vigilance, and sleepiness following sleep deprivation. *Journal of sleep research, 17*(1), 34-41.

Fuchikawa, T., Eban-Rothschild, A., Nagari, M., Shemesh, Y., & Bloch, G. (2016). Potent social synchronization can override photic entrainment of circadian rhythms. *Nature Communications, 7*(1), 1-10.

Furlong, T. M., Vianna, D. M., Liu, L., & Carrive, P. (2009). Hypocretin/orexin contributes to the expression of some but not all forms of stress and arousal. *European Journal of Neuroscience, 30*(8), 1603-1614.

Goel, N., Rao, H., Durmer, J. S., & Dinges, D. F. (2009). Neurocognitive consequences of sleep deprivation. Seminars in neurology,

Gooley, J. J., Lu, J., Fischer, D., & Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience, 23*(18), 7093-7106.

Green, E. W., O'Callaghan, E. K., Hansen, C. N., Bastianello, S., Bhutani, S., Vanin, S., Armstrong, J. D., Costa, R., & Kyriacou, C. P. (2015). Drosophila circadian rhythms in seminatural environments: Summer afternoon component is not an artifact and requires TrpA1 channels. *Proceedings of the National Academy of Sciences, 112*(28), 8702-8707. https://doi.org/doi:10.1073/pnas.1506093112

Grønli, J., Meerlo, P., Pedersen, T. T., Pallesen, S., Skrede, S., Marti, A. R., Wisor, J. P., Murison, R., Henriksen, T. E., & Rempe, M. J. (2017). A rodent model of night-shift work induces short-term and enduring sleep and electroencephalographic disturbances. *Journal of biological rhythms, 32*(1), 48-63.

Grønli, J., Rempe, M. J., Clegern, W. C., Schmidt, M., & Wisor, J. P. (2016). Beta EEG reflects sensory processing in active wakefulness and homeostatic sleep drive in quiet wakefulness. *Journal of sleep research, 25*(3), 257-268. https://onlinelibrary.wiley.com/doi/pdfdirect/10.1111/jsr.12380?download=true

Gulevich, G., Dement, W., & Johnson, L. (1966). Psychiatric and EEG observations on a case of prolonged (264 hours) wakefulness. *Archives of General Psychiatry*.

Hauglund, N. L., Pavan, C., & Nedergaard, M. (2020). Cleaning the sleeping brain–the potential restorative function of the glymphatic system. *Current Opinion in Physiology, 15*, 1-6.

Heier, M. S., Evsiukova, T., Vilming, S., Gjerstad, M. D., Schrader, H., & Gautvik, K. (2007). CSF hypocretin-1 levels and clinical profiles in narcolepsy and idiopathic CNS hypersomnia in Norway. *Sleep, 30*(8), 969-973.

Helfrich-Förster, C. (2000, Apr). Differential control of morning and evening components in the activity rhythm of Drosophila melanogaster--sex-specific differences suggest a different quality of activity. *J Biol Rhythms, 15*(2), 135-154. https://doi.org/10.1177/074873040001500208

Helfrich, C., & Engelmann, W. (1983). Circadian rhythm of the locomotor activity in Drosophila melanogaster and its mutants 'sine oculis' and 'small optic lobes'. *Physiological Entomology, 8*(3), 257-272. https://doi.org/10.1111/j.1365-3032.1983.tb00358.x

Herculano-Houzel, S. (2015). Decreasing sleep requirement with increasing numbers of neurons as a driver for bigger brains and bodies in mammalian evolution. *Proceedings of the royal society B: biological sciences, 282*(1816), 20151853.

Hsieh, S., Li, T.-H., & Tsai, L.-L. (2010). Impact of Monetary Incentives on Cognitive Performance and Error Monitoring following Sleep Deprivation. *Sleep, 33*(4), 499-507. https://doi.org/10.1093/sleep/33.4.499

Huber, R., Felice Ghilardi, M., Massimini, M., & Tononi, G. (2004, 2004/07/01). Local sleep and learning. *Nature, 430*(6995), 78-81. https://doi.org/10.1038/nature02663

Hurd, M. W., Debruyne, J., Straume, M., & Cahill, G. M. (1998, Dec 1). Circadian rhythms of locomotor activity in zebrafish. *Physiol Behav, 65*(3), 465-472. https://doi.org/10.1016/s0031-9384(98)00183-8

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., Mathys, H., Seo, J., Kritskiy, O., Abdurrob, F., Adaikkan, C., Canter, R. G., Rueda, R., Brown, E. N., Boyden, E. S., & Tsai, L.-H. (2016, 2016/12/01). Gamma frequency entrainment attenuates amyloid load and modifies microglia. *Nature, 540*(7632), 230-235. https://doi.org/10.1038/nature20587

Jackson, A. F., & Bolger, D. J. (2014). The neurophysiological bases of EEG and EEG measurement: A review for the rest of us. *Psychophysiology, 51*(11), 1061-1071.

Jelic, V., Johansson, S. E., Almkvist, O., Shigeta, M., Julin, P., Nordberg, A., Winblad, B., & Wahlund, L. O. (2000, 2000/07/01/). Quantitative electroencephalography in mild cognitive impairment: longitudinal changes and possible prediction of Alzheimer's disease. *Neurobiology of Aging, 21*(4), 533-540. https://doi.org/https://doi.org/10.1016/S0197-4580(00)00153-6

Jha, P. K., Bouâouda, H., Kalsbeek, A., & Challet, E. (2021, 2021/04/01/). Distinct feedback actions of behavioural arousal to the master circadian clock in nocturnal and diurnal mammals. *Neuroscience & Biobehavioral Reviews, 123*, 48-60. https://doi.org/https://doi.org/10.1016/j.neubiorev.2020.12.011

Kajikawa, Y., & Schroeder, C. E. (2011). How local is the local field potential? *Neuron, 72*(5), 847-858. https://doi.org/10.1016/j.neuron.2011.09.029

Kalinchuk, A. V., Urrila, A. S., Alanko, L., Heiskanen, S., Wigren, H. K., Suomela, M., Stenberg, D., & Porkka-Heiskanen, T. (2003). Local energy depletion in the basal forebrain increases sleep. *European Journal of Neuroscience, 17*(4), 863-869.

Kang, D., Ding, M., Topchiy, I., & Kocsis, B. (2017). Reciprocal interactions between medial septum and hippocampus in theta generation: Granger causality decomposition of mixed spike-field recordings. *Frontiers in neuroanatomy, 11*, 120.

Kang, J.-E., Lim, M. M., Bateman, R. J., Lee, J. J., Smyth, L. P., Cirrito, J. R., Fujiki, N., Nishino, S., & Holtzman, D. M. (2009). Amyloid-β dynamics are regulated by orexin and the sleep-wake cycle.

Science, *326*(5955), 1005-1007. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2789838/pdf/nihms159243.pdf

Keene, A. C., & Duboue, E. R. (2018, Jun 12). The origins and evolution of sleep. *J Exp Biol, 221*(Pt 11). https://doi.org/10.1242/jeb.159533

Kleitman, N. (1964). The evolutionary theory of sleep and wakefulness. *Perspectives in biology and medicine, 7*(2), 169-178.

Kong, J., Shepel, P. N., Holden, C. P., Mackiewicz, M., Pack, A. I., & Geiger, J. D. (2002). Brain glycogen decreases with increased periods of wakefulness: implications for homeostatic drive to sleep. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 22*(13), 5581-5587. https://doi.org/10.1523/JNEUROSCI.22-13-05581.2002

Lee, M. G., Hassani, O. K., & Jones, B. E. (2005). Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *Journal of Neuroscience, 25*(28), 6716-6720.

Léger, D., Debellemaniere, E., Rabat, A., Bayon, V., Benchenane, K., & Chennaoui, M. (2018). Slow-wave sleep: From the cell to the clinic. *Sleep medicine reviews, 41*, 113-132.

Lesku, J., Roth, T., Amlaner, C., & Lima, S. (2006, 10/01). A Phylogenetic Analysis of Sleep Architecture in Mammals: The Integration of Anatomy, Physiology, and Ecology. *The American Naturalist, 168*, 441-453. https://doi.org/10.1086/506973

Lesku, J. A., Bark, R. J., Martinez-Gonzalez, D., Rattenborg, N. C., Amlaner, C. J., & Lima, S. L. (2008). Predator-induced plasticity in sleep architecture in wild-caught Norway rats (Rattus norvegicus). *Behavioural Brain Research, 189*(2), 298-305. https://doi.org/10.1016/j.bbr.2008.01.006

Lesku, J. A., Rattenborg, N. C., Valcu, M., Vyssotski, A. L., Kuhn, S., Kuemmeth, F., Heidrich, W., & Kempenaers, B. (2012, Sep 28). Adaptive sleep loss in polygynous pectoral sandpipers. *Science, 337*(6102), 1654-1658. https://doi.org/10.1126/science.1220939

Libourel, P.-A., & Barrillot, B. (2020, 2020/06/01/). Is there REM sleep in reptiles? A key question, but still unanswered. *Current Opinion in Physiology, 15*, 134-142. https://doi.org/https://doi.org/10.1016/j.cophys.2020.01.001

Lima, S., Rattenborg, N., Lesku, J., & Amlaner, C. (2005, 10/01). Sleeping under the risk of predation. *Animal Behaviour, 70*, 723-736. https://doi.org/10.1016/j.anbehav.2005.01.008

Liu, Z. W., & Gao, X. B. (2007, Jan). Adenosine inhibits activity of hypocretin/orexin neurons by the A1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. *J Neurophysiol, 97*(1), 837-848. https://doi.org/10.1152/jn.00873.2006

Lundgaard, I., Lu, M. L., Yang, E., Peng, W., Mestre, H., Hitomi, E., Deane, R., & Nedergaard, M. (2017). Glymphatic clearance controls state-dependent changes in brain lactate concentration. *Journal of Cerebral Blood Flow & Metabolism, 37*(6), 2112-2124.

Löw, A., Lang, P. J., Smith, J. C., & Bradley, M. M. (2008). Both predator and prey: Emotional arousal in threat and reward. *Psychological Science, 19*(9), 865-873. https://doi.org/10.1111/j.1467-9280.2008.02170.x

Maloney, K. J., Cape, E., Gotman, J., & Jones, B. (1997). High-frequency γ electroencephalogram activity in association with sleep-wake states and spontaneous behaviors in the rat. *Neuroscience, 76*(2), 541-555.

Marti, A. R., Pedersen, T. T., Wisor, J. P., Mrdalj, J., Holmelid, Ø., Patil, S., Meerlo, P., Bramham, C. R., & Grønli, J. (2020, Aug 4). Cognitive function and brain plasticity in a rat model of shift work: role of daily rhythms, sleep and glucocorticoids. *Sci Rep, 10*(1), 13141. https://doi.org/10.1038/s41598-020-69969-x

Mascetti, G. G. (2016). Unihemispheric sleep and asymmetrical sleep: behavioral, neurophysiological, and functional perspectives. *Nature and science of sleep, 8*, 221-238. https://doi.org/10.2147/NSS.S71970

McGinley, M. J., Vinck, M., Reimer, J., Batista-Brito, R., Zagha, E., Cadwell, C. R., Tolias, A. S., Cardin, J. A., & McCormick, D. A. (2015). Waking state: rapid variations modulate neural and behavioral responses. *Neuron, 87*(6), 1143-1161.

Mileykovskiy, B. Y., Kiyashchenko, L. I., & Siegel, J. M. (2005). Behavioral correlates of activity in identified hypocretin/orexin neurons. *Neuron, 46*(5), 787-798.

Milinski, L., Fisher, S. P., Cui, N., McKillop, L. E., Blanco-Duque, C., Ang, G., Yamagata, T., Bannerman, D. M., & Vyazovskiy, V. V. (2021, 2021/04/06). Waking experience modulates sleep need in mice. *BMC Biology, 19*(1), 65. https://doi.org/10.1186/s12915-021-00982-w

Mirmiran, M., Scholtens, J., Van de Poll, N. E., Uylings, H. B. M., Van der Gugten, J., & Boer, G. J. (1983, 1983/04/01/). Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. *Developmental Brain Research, 7*(2), 277-286. https://doi.org/https://doi.org/10.1016/0165-3806(83)90184-0

Modell, H., Cliff, W., Michael, J., McFarland, J., Wenderoth, M. P., & Wright, A. (2015). A physiologist's view of homeostasis. *Advances in physiology education*.

Monk, T. H., Fookson, J. E., Kream, J., Moline, M. L., Pollak, C. P., & Weitzman, M. B. (1985). Circadian factors during sustained performance: Background and methodology. *Behavior Research Methods, Instruments, & Computers, 17*(1), 19-26.

Moscardo, E., & Rostello, C. (2010). An integrated system for video and telemetric electroencephalographic recording to measure behavioural and physiological parameters. *Journal of Pharmacological and Toxicological Methods, 62*(1), 64-71.

Naumann, A., Bellebaum, C., & Daum, I. (2006). Cognitive deficits in narcolepsy. *Journal of sleep research, 15*(3), 329-338.

Neckelmann, D., Olsen, Ø. E., Fagerland, S., & Ursin, R. (1994). The reliability and functional validity of visual and semiautomatic sleep/wake scoring in the Møll-Wistar rat. *Sleep, 17*(2), 120-131.

Neckelmann, D., & Ursin, R. (1993). Sleep stages and EEG power spectrum in relation to acoustical stimulus arousal threshold in the rat. *Sleep, 16 5*, 467-477.

Oberauer, K., & Lewandowsky, S. (2019, 2019/10/01). Addressing the theory crisis in psychology. *Psychonomic Bulletin & Review, 26*(5), 1596-1618. https://doi.org/10.3758/s13423-019-01645-2

Oken, B. S., Salinsky, M. C., & Elsas, S. (2006). Vigilance, alertness, or sustained attention: physiological basis and measurement. *Clinical neurophysiology, 117*(9), 1885-1901.

Olejniczak, P. (2006). Neurophysiologic Basis of EEG. *Journal of Clinical Neurophysiology, 23*(3), 186-189. https://doi.org/10.1097/01.wnp.0000220079.61973.6c

Oliva, A., Fernández-Ruiz, A., Fermino de Oliveira, E., & Buzsáki, G. (2018, 2018/11/13/). Origin of Gamma Frequency Power during Hippocampal Sharp-Wave Ripples. *Cell Reports, 25*(7), 1693-1700.e1694. https://doi.org/https://doi.org/10.1016/j.celrep.2018.10.066

Palma, J.-A., Urrestarazu, E., & Iriarte, J. (2013, 2013/03/01/). Sleep loss as risk factor for neurologic disorders: A review. *Sleep Medicine, 14*(3), 229-236. https://doi.org/https://doi.org/10.1016/j.sleep.2012.11.019

Parmelee Jr, A. H., Wenner, W. H., & Schulz, H. R. (1964). Infant sleep patterns: from birth to 16 weeks of age. *The Journal of pediatrics, 65*(4), 576-582.

Paukert, M., Agarwal, A., Cha, J., Doze, V. A., Kang, J. U., & Bergles, D. E. (2014). Norepinephrine controls astroglial responsiveness to local circuit activity. *Neuron, 82*(6), 1263-1270.

Pedersen, W. C., Denson, T. F., Goss, R. J., Vasquez, E. A., Kelley, N. J., & Miller, N. (2011). The impact of rumination on aggressive thoughts, feelings, arousal, and behaviour. *British Journal of Social Psychology, 50*(2), 281-301.

Perez-Atencio, L., Garcia-Aracil, N., Fernandez, E., Barrio, L. C., & Barios, J. A. (2018, Jan). A four-state Markov model of sleep-wakefulness dynamics along light/dark cycle in mice. *PLoS One, 13*(1), e0189931, Article e0189931. https://doi.org/10.1371/journal.pone.0189931

Peyron, C., Tighe, D. K., Van Den Pol, A. N., De Lecea, L., Heller, H. C., Sutcliffe, J. G., & Kilduff, T. S. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *Journal of Neuroscience, 18*(23), 9996-10015.

Pilcher, J. J., & Huffcutt, A. I. (1996). Effects of sleep deprivation on performance: a meta-analysis. *Sleep, 19*(4), 318-326.

Porkka-Heiskanen, T., & Kalinchuk, A. V. (2011, Apr). Adenosine, energy metabolism and sleep homeostasis. *Sleep Med Rev, 15*(2), 123-135. https://doi.org/10.1016/j.smrv.2010.06.005

Porkka-Heiskanen, T., Strecker, R. E., Thakkar, M., Bjørkum, A. A., Greene, R. W., & McCarley, R. W. (1997). Adenosine: A Mediator of the Sleep-Inducing Effects of Prolonged Wakefulness. *Science, 276*(5316), 1265-1268. https://doi.org/doi:10.1126/science.276.5316.1265

Poulet, J. F. A., & Crochet, S. (2019). The Cortical States of Wakefulness. *Frontiers in systems neuroscience, 12*, 64-64. https://doi.org/10.3389/fnsys.2018.00064

Rattenborg, N. C., de la Iglesia, H. O., Kempenaers, B., Lesku, J. A., Meerlo, P., & Scriba, M. F. (2017). Sleep research goes wild: new methods and approaches to investigate the ecology, evolution

and functions of sleep. *Philosophical Transactions of the Royal Society B: Biological Sciences, 372*(1734), 20160251.

Rattenborg, N. C., Voirin, B., Vyssotski, A. L., Kays, R. W., Spoelstra, K., Kuemmeth, F., Heidrich, W., & Wikelski, M. (2008). Sleeping outside the box: electroencephalographic measures of sleep in sloths inhabiting a rainforest. *Biology Letters, 4*(4), 402-405. https://doi.org/doi:10.1098/rsbl.2008.0203

Rechtschaffen, A., Hauri, P., & Zeitlin, M. (1966, Jun). Auditory awakening thresholds in REM and NREM sleep stages. *Percept Mot Skills, 22*(3), 927-942. https://doi.org/10.2466/pms.1966.22.3.927

Rempe, M. J., & Wisor, J. P. (2015). Cerebral lactate dynamics across sleep/wake cycles. *Frontiers in computational neuroscience, 8*, 174. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4294128/pdf/fncom-08-00174.pdf

Sadigh-Eteghad, S., Sabermarouf, B., Majdi, A., Talebi, M., Farhoudi, M., & Mahmoudi, J. (2015). Amyloid-beta: a crucial factor in Alzheimer's disease. *Medical principles and practice, 24*(1), 1-10.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., & Wilson, S. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell, 92*(4), 573-585.

Savage, V. M., & West, G. B. (2007). A quantitative, theoretical framework for understanding mammalian sleep. *Proceedings of the National Academy of Sciences, 104*(3), 1051-1056.

Schober, J. M., & Pfaff, D. (2007). The neurophysiology of sexual arousal. *Best practice & research Clinical endocrinology & metabolism, 21*(3), 445-461.

Siegel, J. M. (2005). Clues to the functions of mammalian sleep. *Nature, 437*(7063), 1264-1271. https://www.nature.com/articles/nature04285

Siegel, J. M. (2009). Sleep viewed as a state of adaptive inactivity. *Nature Reviews Neuroscience, 10*(10), 747-753.

Silber, M. H., Ancoli-Israel, S., Bonnet, M. H., Chokroverty, S., Grigg-Damberger, M. M., Hirshkowitz, M., Kapen, S., Keenan, S. A., Kryger, M. H., Penzel, T., Pressman, M. R., & Iber, C. (2007, Mar 15). The visual scoring of sleep in adults. *J Clin Sleep Med, 3*(2), 121-131.

Simasko, S. M., & Mukherjee, S. (2009, 2009/01/23/). Novel analysis of sleep patterns in rats separates periods of vigilance cycling from long-duration wake events. *Behavioural Brain Research, 196*(2), 228-236. https://doi.org/https://doi.org/10.1016/j.bbr.2008.09.003

Sofroniew, M. V., & Vinters, H. V. (2010). Astrocytes: biology and pathology. *Acta neuropathologica, 119*(1), 7-35. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2799634/pdf/401_2009_Article_619.pdf

Stephan, F. K. (2002). The “other” circadian system: food as a Zeitgeber. *Journal of biological rhythms, 17*(4), 284-292. https://journals.sagepub.com/doi/pdf/10.1177/074873040201700402

Szabó, Z., Héja, L., Szalay, G., Kékesi, O., Füredi, A., Szebényi, K., Dobolyi, Á., Orbán, T. I., Kolacsek, O., & Tompa, T. (2017). Extensive astrocyte synchronization advances neuronal coupling in slow wave activity in vivo. *Scientific reports, 7*(1), 1-18.

Tahara, Y., Aoyama, S., & Shibata, S. (2017, Jan). The mammalian circadian clock and its entrainment by stress and exercise. *J Physiol Sci, 67*(1), 1-10. https://doi.org/10.1007/s12576-016-0450-7

Takahashi, K., Lin, J. S., & Sakai, K. (2008, 2008/05/15/). Neuronal activity of orexin and non-orexin waking-active neurons during wake–sleep states in the mouse. *Neuroscience, 153*(3), 860-870. https://doi.org/https://doi.org/10.1016/j.neuroscience.2008.02.058

Takata, N., Mishima, T., Hisatsune, C., Nagai, T., Ebisui, E., Mikoshiba, K., & Hirase, H. (2011). Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity in vivo. *Journal of Neuroscience, 31*(49), 18155-18165.

Tingley, D., McClain, K., Kaya, E., Carpenter, J., & Buzsáki, G. (2021, 2021/09/01). A metabolic function of the hippocampal sharp wave-ripple. *Nature, 597*(7874), 82-86. https://doi.org/10.1038/s41586-021-03811-w

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep medicine reviews, 10*(1), 49-62.

Tsujino, N., & Sakurai, T. (2013). Role of orexin in modulating arousal, feeding, and motivation. *Frontiers in behavioral neuroscience, 7*, 28.

Ursin, R., & Larsen, M. (1983, 1983/09/30/). Increased sleep following intracerebroventricular injection of the delta sleep-inducing peptide in rats. *Neuroscience Letters, 40*(2), 145-149. https://doi.org/https://doi.org/10.1016/0304-3940(83)90293-8

Van Dongen, H., Maislin, G., Mullington, J. M., & Dinges, D. F. (2003). The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep, 26*(2), 117-126.

Vassalli, A., & Franken, P. (2017). Hypocretin (orexin) is critical in sustaining theta/gamma-rich waking behaviors that drive sleep need. *Proceedings of the National Academy of Sciences, 114*(27), E5464-E5473.

Vassalli, A., & Franken, P. (2017, Jul 3). Hypocretin (orexin) is critical in sustaining theta/gamma-rich waking behaviors that drive sleep need. *Proc Natl Acad Sci U S A, 114*(27), E5464-E5473. https://doi.org/10.1073/pnas.1700983114

Vyazovskiy, V. V. (2015). Sleep, recovery, and metaregulation: explaining the benefits of sleep. *Nature and science of sleep, 7*, 171.

Vyazovskiy, V. V., Achermann, P., & Tobler, I. (2007, 2007/09/14/). Sleep homeostasis in the rat in the light and dark period. *Brain Research Bulletin, 74*(1), 37-44. https://doi.org/https://doi.org/10.1016/j.brainresbull.2007.05.001

Vyazovskiy, V. V., Cirelli, C., Tononi, G., & Tobler, I. (2008, 2008/03/28/). Cortical metabolic rates as measured by 2-deoxyglucose-uptake are increased after waking and decreased after sleep in

mice. *Brain Research Bulletin, 75*(5), 591-597. https://doi.org/https://doi.org/10.1016/j.brainresbull.2007.10.040

Vyazovskiy, V. V., Olcese, U., Hanlon, E. C., Nir, Y., Cirelli, C., & Tononi, G. (2011). Local sleep in awake rats. *Nature, 472*(7344), 443-447.

Vyazovskiy, V. V., & Tobler, I. (2005, 2005/07/19/). Theta activity in the waking EEG is a marker of sleep propensity in the rat. *Brain Research, 1050*(1), 64-71. https://doi.org/https://doi.org/10.1016/j.brainres.2005.05.022

Wang, G., Harpole, C. E., Trivedi, A. K., & Cassone, V. M. (2012, Apr). Circadian regulation of bird song, call, and locomotor behavior by pineal melatonin in the zebra finch. *J Biol Rhythms, 27*(2), 145-155. https://doi.org/10.1177/0748730411435965

Waters, F., Chiu, V., Atkinson, A., & Blom, J. D. (2018, 2018-July-10). Severe Sleep Deprivation Causes Hallucinations and a Gradual Progression Toward Psychosis With Increasing Time Awake [Review]. *Frontiers in Psychiatry, 9*. https://doi.org/10.3389/fpsyt.2018.00303

Whitmore, D., Cermakian, N., Crosio, C., Foulkes, N. S., Pando, M. P., Travnickova, Z., & Sassone-Corsi, P. (2000). A clockwork organ.

Wigren, H. K., Rytkönen, K. M., & Porkka-Heiskanen, T. (2009, Sep 16). Basal forebrain lactate release and promotion of cortical arousal during prolonged waking is attenuated in aging. *J Neurosci, 29*(37), 11698-11707. https://doi.org/10.1523/jneurosci.5773-08.2009

Wisor, J. P., Rempe, M. J., Schmidt, M. A., Moore, M. E., & Clegern, W. C. (2013, Aug). Sleep slow-wave activity regulates cerebral glycolytic metabolism. *Cereb Cortex, 23*(8), 1978-1987. https://doi.org/10.1093/cercor/bhs189

Wu, H., Dunnett, S., Ho, Y.-S., & Chang, R. C.-C. (2019, 2019/07/01/). The role of sleep deprivation and circadian rhythm disruption as risk factors of Alzheimer's disease. *Frontiers in Neuroendocrinology, 54*, 100764. https://doi.org/https://doi.org/10.1016/j.yfrne.2019.100764

Xia, T., & Law, L. A. F. (2008). A theoretical approach for modeling peripheral muscle fatigue and recovery. *Journal of biomechanics, 41*(14), 3046-3052.

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., & Iliff, J. J. (2013). Sleep drives metabolite clearance from the adult brain. *Science, 342*(6156), 373-377.

Yamanaka, Y., Honma, K.-i., Hashimoto, S., Takasu, N., Miyazaki, T., & Honma, S. (2006). Effects of physical exercise on human circadian rhythms. *Sleep and Biological Rhythms, 4*(3), 199-206.

Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., & Tomioka, K. (2004, Jun). Drosophila cryb mutation reveals two circadian clocks that drive locomotor rhythm and have different responsiveness to light. *J Insect Physiol, 50*(6), 479-488. https://doi.org/10.1016/j.jinsphys.2004.02.011

Young, C. K., & McNaughton, N. (2008). Coupling of Theta Oscillations between Anterior and Posterior Midline Cortex and with the Hippocampus in Freely Behaving Rats. *Cerebral Cortex, 19*(1), 24-40. https://doi.org/10.1093/cercor/bhn055

Zeeuw, J. d., Wisniewski, S., Papakonstantinou, A., Bes, F., Wahnschaffe, A., Zaleska, M., Kunz, D., & Münch, M. (2018, 2018/07/20). The alerting effect of the wake maintenance zone during 40 hours of sleep deprivation. *Scientific reports, 8*(1), 11012. https://doi.org/10.1038/s41598-018-29380-z

Zielinski, M. R., & Krueger, J. M. (2011). Sleep and innate immunity. *Frontiers in bioscience (Scholar edition), 3*, 632.

Zielinski, M. R., McKenna, J. T., & McCarley, R. W. (2016). Functions and Mechanisms of Sleep. *AIMS Neurosci, 3*(1), 67-104. https://doi.org/10.3934/Neuroscience.2016.1.67

Zoccoli, G., & Amici, R. (2020). Sleep and autonomic nervous system. *Current Opinion in Physiology, 15*, 128-133.

Åkerstedt, T., & Folkard, S. (1997). The three-process model of alertness and its extension to performance, sleep latency, and sleep length. *Chronobiology international, 14*(2), 115-123.