Testing predictions from the synaptic homeostasis hypothesis: A sleep deprivation study of cortical thickness and visual evoked potentials

Linn Christin Bonaventure Norbom

Master of Philosophy in Psychology,

Cognitive Neuroscience discipline at the Department of Psychology.

UNIVERSITY OF OSLO

May 2013

Year: 2013

Title: Testing predictions from the synaptic homeostasis hypothesis: A sleep deprivation study of cortical thickness and visual evoked potentials

Author: Linn Christin Bonaventure Norbom

http://www.duo.uio.no/

Print: Reprosentralen, University of Oslo

Acknowledgements

The current thesis was motivated by a great interest in sleep and sleep function. As none of the professors I contacted viewed this as their main field of expertise I was uncertain if I should pursue sleep as a topic for neuroscientific investigation. I therefore became very excited when I learned that I could be a research assistant on a sleep deprivation study at Oslo University Hospital, Rikshospitalet. My tasks were to collect most of the VEP and sMRI data and chaperone 12 of the sleep deprived subjects overnight. Additionally, I analyzed all the structural data at the Neuroimaging Analysis Lab at KG Jebsen center for psychosis research, and performed statistical analysis of VEP data.

The greatest acknowledgments are reserved for my supervisor Lars Tjelta Westlye. I sincerely thank you for a great collaboration on writing my master thesis. I truly appreciate the patience and guidance through each and every step. Additional thanks for reading through my paper several times and for thorough training in both Linux and sMRI analysis. Thank you for always answering my mails or knocks on your office door no matter the hour or day.

I would like to additionally sincerely thank Torbjørn Elvsåshagen for allowing me to be a part of his project and for thorough training in both VEP and sMRI collection. I would also like to thank you for reading through my paper and aiding me on the notion of plasticity. Additional thanks to Torgeir Moberget for analyzing VEP data and to Grethe Løvland and Svein Are Vatnehol for teaching scanner safety and general procedures concerning scanning. I would also like to thank the other research assistant Per Øystein Heiberg Pedersen for a great collaboration during data collection. Additional thanks to my fellow student Luigi Maglanoc who aided me during difficult times caused by Linux and FreeSurfer and to Even Fagerli for helping me to improve SPSS graphs in Photoshop.

The experience of conducting and writing about my own research for the first time will be an educational memory I will never forget. The remaining mistakes are mine and mine alone.

Abstract

Although there is no shortage on theories, the issue of sleep function remains one of the open questions within neuroscience today. In the current study predictions from a theory of synaptic plasticity was tested through a sleep deprivation study of cortical thickness and visual evoked potentials. In accordance with our initial hypothesis we report that wake foster measurable macrostructural increases in grey matter presented as 4 bilateral clusters. This increase was not significantly alternated during a period of sleep deprivation. Thus our results support a prominent theory linking sleep to plastic processes, with the findings that waking increase synaptic potentiation and that sleep is needed for synaptic regulation.

Table of contents

1.0 Introduction

On the evening of March 23rd 1989, a ship carrying over 200 000 tons of oil started its journey from Alaska to California. The ship called Exxon Valdez was almost brand new but had already embarked on this route several times before. Around midnight an exhausted third mate named Gregory Cousins was controlling the ship alone, against company rules. Second mate Lloyd LeCain was scheduled to have relieved him already, but after a gruelling dayshift LeCain was still sleeping in his room. Cousins was aware of his colleague's desperate need for rest and decided not to wake him. Instead he remained on duty himself after approximately 18 hours of physical and stressful labour. Some time after midnight the massive vessel hit a reef, which ripped through large portions of the ship. It has been estimated that between 40 000 and 100 000 tons of oil leaked into the ocean that night. The details of Cousins' state has been debated, but after only 4 hours of preceding sleep Cousins was either too cognitively impaired to manage the vessel properly, or worse, he might even have fallen asleep. Tiresome overtime without compensatory sleep contributed to one of the largest manmade environmental disasters in history (Adasiak, Ricbter, & Spivey, 1990).

As exemplified above sleep loss has direct effects on cognitive abilities. Indeed, the cognitive setbacks of the sleep-deprived brain have been documented time and again, and especially within the field of neuroscience. Lack of sleep can have detrimental effects on attention, memory, mood and even decision making to name a few (Durmer & Dinges, 2005). Although it is quite clear that sleep offers cognitive as well as physical and mental (Reid et al., 2006) benefits, its function remain somewhat of a mystery. It is quite peculiar that humans in total spend about 1/3 of their lives asleep (Siegel, 2005), and scientific enquiries have provided little if any knowledge as to why.

The question of sleep function has been of interest since the time of the ancient philosophers, if not earlier. As Aristotle noticed that sleepiness was brought on by meals, he concluded in his text on sleep and dreaming that humans and animals sleep to digest food (Wijsenbeek-Wijler, 1976). Centuries later, in 1896 the first study that explored sleep deprivation in conjunction with cognition was published. The study involved an observation of three participants who were sleep deprived for 90 hours consecutively. The researchers reported a decline in several motor and memory skills (Durmer & Dinges, 2005). Although it has been difficult to date the first study claiming there is a relationship between sleep and memory, the article "Obliviscence during sleep and waking" from 1924 is often mentioned among the earliest (Jenkins & Dallenbach, 1924). Indeed, the "memory processing-function"

of sleep is still an attractive notion and has been since the 1960's (Vertes & Eastman, 2000). Today, mechanisms of synaptic plasticity are being investigated in conjunction with sleep to get new insight to its possible connections to memory (Benington & Frank, 2003). Another milestone within sleep research was the discovery of "slow wave sleep" (SWS) in 1937. This was achieved by the use of electroencephalography (EEG), a method that continues to be pivotal within sleep research today (Blake & Gerard, 1937). In 1953 Aserinskey and Kleitmann discovered another intriguing sleep state named rapid eye movement (REM)-sleep after its most prominent feature (Sandyk, Tsagas, Anninos, & Derpapas, 1992). As several subcomponents of sleep have been discovered, researchers have started to consider if these components also serve different functions.

What is the function of sleep? Although there is no shortage on theories, this issue remains one of the open questions within neuroscience today. In the following, the function of sleep will be explored through a theory of synaptic plasticity. This theory suggests that synapses are potentiated during the day and that sleep is crucial for bringing potentiated synapses back to a homeostatic level (Tononi & Cirelli, 2006). Possible evidence for this theory will be demonstrated through a sleep deprivation study and by the use of structural magnetic resonance imaging (sMRI) and visual evoked potentials (VEP).

1.1 Aspects of sleep that support sleep function

Before examining sleep function one should clarify why scientists are so confident that sleep serves a function at all. Several key aspects of sleep have been revealed by a variety of research, promoting the understanding that sleep is not a mere epiphenomenon.

From an evolutionary perspective sleep can in several ways be regarded as maladaptive since animals cannot mate, take care of offspring or acquire food during periods of sleep. Also, the sleep-state is a vulnerable state where one is less responsive to stimuli, and unable to protect oneself from predators (Siegel, 2012). Sleep does on the other hand have positive aspects as well. Sleep saves energy, slows metabolism and prevents animals from being active when it is not productive (Siegel, 2009). Nevertheless, numerous researchers believe the functions of sleep extend beyond these facets, thus arguing that although sleep occurs in the most profitable timeframe it does not automatically make it the sole purpose of sleep. In addition, although debated, several prominent sleep researchers state that sleep is in fact universal (Gilestro, Tononi, & Cirelli, 2009). This means that all animals including insects sleep. Moreover sleep is believed to be both pervasive (Bergh & Mulder, 2012) and irresistible (Orzel-Gryglewska, 2010). One could therefore argue that, despite the

disadvantages, sleep serves several functions that cannot be accomplished as effectively in any other state in any animal (Azar, 2006).

As mentioned, cognitive research has not been sparse when it comes to demonstrating the limitations of the sleep-deprived brain. In general, converging results strongly support that sleep loss is detrimental for most cognitive tasks (Lim & Dinges, 2010). In extreme circumstances, total sleep deprivation can cause delusions and hallucinations (Ross, 1965). Moreover, rats have been reported to die after some weeks of total sleep loss (Rechtschaffen & Bergmann, 1995). Indeed, it has been argued that the same outcome is expected in humans after long-term sleep deprivation. Fatal familial insomnia, a rare disease characterised by progressive untreatable insomnia, results in death after several months (Almer et al., 1999). Still, whether death is directly or indirectly caused by sleep deprivation is disputable.

The features described above demonstrate that sleep most likely serve an important purpose. There is far less agreement however upon *what* is actually aided by sleeping. Nevertheless, Allan Rechtschaffen captured the current belief within sleep research noting that: "If sleep doesn't serve an absolutely vital function, it is the greatest mistake evolution ever made" (Stickgold, 2006, p. 559).

1.2 Sleep definition, architecture and characteristics

At the behavioural level sleep is usually defined by specific postures which vary across species, behavioural inactivity, elevated arousal threshold, rebound if deprived, and reversed if stimulated (Vassalli & Dijk, 2009). Within the neurosciences, sleep is often defined in terms of electrophysiological characteristics. These characteristics can easily be detected by the use of polysomnography, which is a conjunction of measurements usually comprising an EEG and an electro-oculography (EOG), which measures eye movements, which is important for detection of REM-sleep. In addition, one usually includes a measure of chin movement and body muscle and hart rate (Taheri, Lin, Austin, Young, & Mignot, 2004).

Sleep is today understood as consisting of two distinct forms. These are called REM- and non-REM (NREM)-sleep. NREM sleep can be further subdivided into four different stages, roughly corresponding to sleep depth. Stage 1-sleep is the lightest form of sleep, which occurs immediately after sleep onset or what subjectively might feel like "falling asleep". Stage 2 is another form of light sleep, characterized by bursts of brain activity or what is called sleep spindles. Stage 3 and 4 is characterised by low frequency high amplitude (delta) waves and is therefore in conjunction referred to as slow wave sleep. SWS is the deepest form of sleep and correspondingly responsiveness threshold is elevated during this state (Carskadon

& Dement, 2011). It is believed that the thalamus and the cerebral cortex are key neuroanatomical components of the bioelectric and behavioral aspects of NREM sleep. Other structures are also central such as frontal brain areas, parts of the hypothalamus and brain stem, cerebellum, spinal cord as well as parts of the autonomic nervous system (Andrés, Garzón, & Reinoso-Suárez, 2011).

REM-sleep, which is distinct from the other four sleep types, is often referred to as the fifth stage. Although not characterised as light sleep per se, REM brain activity (theta waves) actually resembles waking activity. The REM state has several additional characteristics such as bursts of rapid eye movements, total body paralysis called atonia and frequent and vivid dreaming (Carskadon & Dement, 2011). The pontine tegmentum is central for the generation of REM sleep (Braun et al., 1997). In addition, individual signs of REM sleep can be evoked by different brainstem structures (Reinoso-Suarez, Andres, Rodrigo-Angulo, & Garzon, 2001). A human night of sleep usually consists of 90-minute cycles, where one fluctuates between the different sleep stages. During the first part of the night when sleep pressure is high there is commonly a high prevalence of slow wave sleep. In the later parts of the night when sleep pressure declines, REM sleep becomes increasingly central (Feinberg & Floyd, 1979)

1.3 Plasticity

According to a prominent sleep theory that later will be discussed in detail, sleep could be connected to plastic processes within the cortex (Tononi & Cirelli, 2006). Contrary to earlier beliefs, one today regards the adult brain as a plastic entity, meaning it has the ability to change over time in response to environmental demands (Eriksson et al., 1998). This change can be manifested at different levels in terms of structural and functional alterations. As changes in neuronal function are essentially always based on a structural change at some level, the clear distinction between structural and functional plasticity is at times hard to make (Castren & Hen, in press). Macrostructural neuroanatomical changes can be detected in humans by employing advanced sMRI techniques typically coupled with motor- or cognitive training, allowing for quantification of e.g. cortical grey matter changes (Draganski et al., 2004).

Synaptic plasticity is a leading candidate mechanism for learning (Derrick, 2007). The most prevalent forms of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD). LTP can be defined as a persistent enhancement of synaptic signal transmission, while LTD can be defined as a persistent weakening of synaptic signal

transmission (Bliss & Lømo, 1973). LTP can be divided into earlier and later phases depending on the progress of change. Early LTP (E-LTP) can occur after only a few hours by for example a postsynaptic neuron attaining additional receptors (Bliss & Cooke, 2011). Neurogenesis on the other hand, is the creation of a new functional nerve cell. This, and other protein synthesis-dependent mechanisms are examples of more robust and long-lasting forms of LTP (Bliss & Cooke, 2011). These processes could take several days if not weeks and are thus understood as late LTP (L-LTP). It has been postulated that sleep might enable the plastic processes described to continue efficiently across time (Tononi & Cirelli, 2006).

2.0 The SHY hypothesis

The synaptic homeostasis hypothesis (SHY) is an intriguing theory on sleep function formally proposed by Tononi and Cirelli in 2003 (Tononi & Cirelli, 2003). It attempts to explain the function of slow wave sleep by connecting it to a process of synaptic downscaling. A positive aspect of this theory is that it strives to be consistent with one of the most established theories within sleep research today, namely the "Two-process model". The two process model is a description of sleep regulation consisting of a "process S": homeostatic sleep drive and a "process C": circadian regulation (Borbély & Tononi, 1998). Tononi and Cirelli state that SHY is a detailed description of "process S" (Tononi & Cirelli, 2006).

A schematic illustration of SHY is provided in Figure 1. The basic assumptions of SHY are that wakefulness result in a net increase in synaptic strength. Synaptic potentiation increases the metabolic burden, and slow wave sleep is therefore needed to downscale synapses back to a sustainable level (Tononi & Cirelli, 2006). In a way sleep is the price we pay for plasticity" (Tononi & Cirelli, 2012).

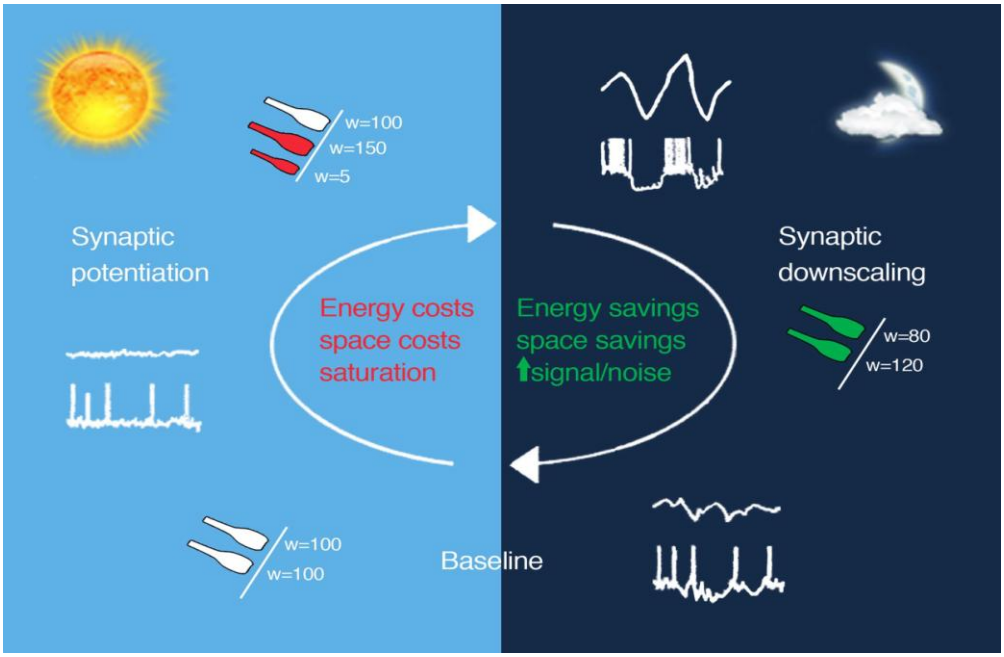

Figure 1. Schematic illustration of SHY.

During wakefulness (light background) humans experience the environment. Synapses (in white) are shown with an arbitrary starting weight of 100 and neurons spike as within normal waking which result in a corresponding "waking-EEG". As the day comes to an end one synapse (indicated in red) has grown to a weight of 150, while the second synapse remains unchanged. In addition, a new synapse with a weight of five (indicated in red) has appeared. During succeeding sleep (dark background) synchronous neuronal firing which results in large slow waves are reflected in the EEG. Synaptic downscaling commences and synapses are weakened by (e.g.) 20% (green color). The synapse with a weight of 5 has been downscaled below minimum strength and removed. The reduced synaptic strength also reduces the synchronization of neuronal activity as reflected in less slow wave activity in the EEG. As a new day commences, neural circuits have preserved traces from previous experiences, but are at the same time ready for the cycle to start over. Figure adapted from (Tononi & Cirelli, 2006).

2.1. Wakefulness foster synaptic potentiation

The awake-state consists of continuous stimulation from the environment. According to SHY this stimuli, be it active learning or "passive involvement", will result in plastic changes at the synaptic level (Tononi & Cirelli, 2006). These changes are predominantly caused by presynaptic firing, followed by depolarization, that is; firing of postsynaptic neurons (Tononi & Cirelli, 2006). In addition the neuromodulatory milieu (for example high levels of noradrenaline) will aid the storage of what is registered as a significant event (Tononi & Cirelli, 2006). In other words synaptic plasticity will mainly be caused by LTP rather than by LTD. Consequently, a full day of waking will generate a net increase in synaptic strength throughout large portions of the cortex (Tononi & Cirelli, 2006).

2.1.1 Evidence for wakefulness fostering synaptic potentiation

Much of the supporting evidence for wake fostering synaptic potentiation derives from anatomical animal studies. Knott and colleagues (Knott, Quairiaux, Genoud, & Welker, 2002) investigated plastic processes within the adult cortex by performing a whisker stimulation study on adult mice. A metal piece was fastened onto a single whisker, leaving the others untouched. The whisker was then stimulated in a way that resembled normal whisker activity by an electromagnetic coil. Synaptic density was then calculated by serial electron micrographs, in addition to a "blind" observer counting synapses. The researchers discovered that after stimulating the whisker for 24 hours, synaptic density in the corresponding brain area had increased by 36%. The increase was concluded to be caused by an escalation of synapses, both excitatory and inhibitory (Knott et al., 2002). It should be mentioned that the current article is not clear about potential sleep episodes within the experiment. Although the mice were "freely moving" and continuously stimulated, this is not necessarily an effective deprivation method in itself. As the stimulation was not painful, one cannot eliminate the possibility that as mice got progressively tired they were able to sleep while the whisker was stimulated.

Another rodent study also concluded in favor of SHY. Cirelli and colleagues investigated gene expression within the sleep-wake cycle in rats and reported that wakefulness (independent of time a day) was associated with molecular changes associated with LTP such as Arc and BDNF. Sleep on the other hand was shown to severely reduce or even eliminate the expression of LTP-related genes, while at the same time fostering protein synthesis and other characteristics of synaptic depression (Cirelli, Gutierrez, & Tononi, 2004).

Drosophila melanogaster more commonly known as the fruit fly is a popular subject within sleep research. Although the drosophila brain is very different from mammalian brains, numerous sleep characteristics are shared. In addition, drosophila have similar plastic mechanisms and synaptic features as humans (Gilestro et al., 2009). Gilestro and colleagues investigated whether waking and sleep affected synaptic markers within the drosophila central nervous system. Bruchpilot protein levels, which are important for synaptic activity, were used to quantify synapse number. Male flies were either sleep deprived by a mechanical method, or were allowed undisturbed sleep. After the deprivation period brains were dissected. The researchers found that bruchpilot levels were higher after sleep deprivation compared to sleeping controls. This increase correlated with the duration of the sleep deprivation. Correspondingly, volumetric increases where located in antennal lobes of sleep deprived flies. Although ultimately indirect the observed increases in either number or volume

of synaptic connections occurring during wakefulness (Gilestro et al., 2009), is evidence in favor of SHY. An additional study also investigating the fruit fly, attempted to find direct evidence for wake and sleep fostering morphological changes within synaptic terminals (Bushey, Tononi, & Cirelli, 2011). The researchers selected specific cell populations within the fly brain and proposed that wake might affect synaptic density and size. Adult flies were either sleep deprived or were able to sleep undisturbed. A researcher blind to the conditions then imaged the brains with a Prairie confocal microscope. The results yielded a volume increase in the presynaptic terminals of certain cells after sleep deprivation. In other cells the increase was manifested in spine number, branching and lengthening of dendritic trees. In addition, richer waking experiences resulted in larger synaptic growth (Bushey et al., 2011).

Human research although indirect has also been in concordance with SHY. A PET study by Braun and colleagues reported that metabolism increased from early to late wakefulness (Braun et al., 1997). Since brain metabolism as measured by PET likely reflects synaptic activity, and since potentiated synapses require more energy, these results are in concordance with SHY (Tononi & Cirelli, 2006).

2.2 Sleep and synaptic downscaling

Although there are immense adaptive and cognitive benefits to a plastic brain, it is understood by SHY that this plasticity also comes with several costs. Tononi and Cirelli state that stronger synapses consume more energy, cellular supplies, space and in the end also saturate learning ability. It is reasoned that as more synapses are strengthened, firing threshold is lowered and neurons will become increasingly excitable. Distinguishing actual signal from irrelevant noise will therefore become increasingly difficult. Thus, net potentiation will ultimately decrease the signal to noise ratio. Granted the principles described are correct, the increase in synaptic strength cannot go on indefinitely. Synapses must be regulated and returned to a sustainable level. For cognitive and adaptive reasons this downscaling is according to SHY best achieved during sleep. Synaptic homeostasis or net downscaling is accomplished by reducing the strength of all synapses converging onto the same neuron (Tononi & Cirelli, 2006). In this way it is understood as somewhat different from LTD or de-potentiation. Possibly pruning or removal of weak synapses may also occur (Tononi & Cirelli, 2006). If each synapse discards the same amount of weight the relative difference between them will be preserved and memory traces would ultimately be maintained. (Tononi & Cirelli, 2006).

2.2.1 Evidence for downscaling of synapses during sleep

Supporting evidence for the notion that SWS downscales synapses largely derives from animal studies. In the drosophila study by Gilestro and colleagues previously described, the researchers additionally sought to investigate the decline in bruchpilot expression during sleep. Flies were collected after a day of natural wakefulness, and additionally every 3 hours across the night while sleeping. In both males and females, it was reported that bruchpilot levels declined progressively in the course of sleep, ultimately reaching its lowest level at the end of the night (Gilestro et al., 2009). Although supportive of SHY, one could argue that similar results could have occurred if flies were allowed to rest while awake.

Indeed, in additional experiments from the study by Bushey and colleagues previously described, wakeful rest was implemented as an independent variable. In these experiments the authors focused on morphological changes during sleep. Fruit flies were exposed to what is called a "fly mall", a place where flies could fly around, explore and interact with other flies. Flies were then collected either straight after a day of enriched experience, or after additional hours of sleep deprivation. The researchers reported that sleep deprived flies had similar branch alterations of length and number as flies collected immediately after enrichment. Thus, quiet wake did not downscale the alternated branches. In contrast, flies that were allowed to sleep after the enriched experience had reversed all morphological parameters back to levels observed in waking controls (not experiencing enrichment). Additionally, post sleep recordings revealed that flies that had been active in the fly mall for 12 hours slept more, both during the day and at night (Bushey et al., 2011). Possibly there was an additional need for downscaling as the rich waking experience had resulted in larger increases in synaptic strength.

Rodent studies have also been concordant with the homeostatic proposition of SHY. Liu and colleagues sought to find direct evidence for variations in synaptic strength across the sleep-wake cycle. More precisely they examined if a net increase in synaptic strength would occur after wake and weather this potential increase would be counteracted by sleep. Recordings of miniature excitatory postsynaptic potentials (mEPSCs) from frontal cortex brain slices of rats were examined for frequency (presynaptic modifications) and amplitude (postsynaptic modifications) changes. Rats were either sleep deprived by gentle handling or were allowed to sleep without interruption for 4 hours. Rats were subsequently euthanized and the synaptic efficacy of pyramidal neurons was measured. It was reported that the frequency of mEPSCs was significantly higher in rats kept awake for 4 hours compared to sleeping controls. In addition, the mean amplitude of mEPSCs was also larger in sleep

deprived rats. To investigate the matter further, another experiment was conducted were certain rats were allowed "recovery sleep" of 2 hours after sleep deprivation, while others were not (Liu, Faraguna, Cirelli, Tononi, & Gao, 2010). EEG was employed to record sleep architecture and length. The rats that were allowed to recover after deprivation had significantly lower mEPSCs frequencies compared to deprived controls. Liu and colleagues consequently concluded that synaptic strength increases with continuous wake, affecting both presynaptic and postsynaptic sites. The net synaptic strength in rat frontal cortex is then decreased with recovery sleep. These findings according to the authors is the first direct demonstration of cortical synaptic increases within periods of wake and restoration to lower levels after periods of sleep (Liu et al., 2010), and therefore serve as highly supporting evidence for SHY.

2.3 Slow wave activity, synaptic strength and homeostatic regulation

During NREM sleep most cortical neurons engage in periods of depolarized up states characterized by extensive neuronal firing. These periods are followed by hyperpolarized down states characterized by neuronal silence (Vyazovskiy et al., 2009). Nevertheless, according to SHY, the neuromodulatory milieu (for example low levels of noradrenaline) ensures that synaptic activity does not generate synaptic potentiation (Tononi & Cirelli, 2006). Tononi and Cirelli state that synaptic strength and number influence nighttime firing periods, and the architecture of the slow waves within SWS. Stronger synapses directly increase neuronal firing synchrony, that is the number of neurons experiencing an up (firing)- or down (not firing)-state at the same time (Tononi & Cirelli, 2012). Thus, compared to weaker synapses, highly potentiated ones would give rise to an EEG recording of larger amplitude waves with steeper slopes. Following this argumentation it would also mean that slow wave activity (SWA) would be higher at sleep onset compared to at the end of sleep. Additionally, SHY states that SWA can be locally regulated within the cortex. Accordingly, if synapses within visual cortex is especially potentiated during wake, post sleep EEG recordings should reveal a local SWA increase within visual cortex (Tononi & Cirelli, 2012).

A perhaps bold declaration of SHY is that sleep slow waves may not simply reflect synapse strength and number but may also have an active role in bringing forward synaptic homeostasis (Tononi & Cirelli, 2012). Hence, slow waves are not understood as an epiphenomenon, instead the sequences of depolarization-hyperpolarization is believed to cause the downscaling of potentiated synapses (Tononi & Cirelli, 2006).

2.3.1 Evidence for synaptic strength affecting SWA

In a sleep deprivation study Vyazovskiy and colleagues tested whether cortical neurons fire differently in sleep depending on time awake. Rats were implanted with microwire arrays in the left barrel or frontal cortex, in addition to undergoing a polysomnographic test. All rats were sleep deprived for 4 hours by presenting new objects or tapping the cage if EEG showed drowsiness or rats laid down in sleeping position. After deprivation, rats were allowed rebound sleep. The wake data indicated that as rats were sleep deprived neuronal firing rates increased along with increasing sleep pressure. Post deprivation sleep data revealed that during early NREM sleep neuronal up states were frequent, short, and in addition neurons stopped or resumed firing in near synchrony with the rest of the population. Neuronal down states were also frequent but longer. These findings correlated with changes in SWA, as early sleep was associated with large slow waves with steeper slopes. As sleep progressed firing rates and synchrony decreased, while neuronal up-state periods increased. As expected large slow waves within this time period were rare. The researchers concluded that increases of firing during wake affect SWA and is counterbalanced by sleep (Vyazovskiy et al., 2009).

To investigate possible local qualities of sleep regulation Huber and colleagues performed an EEG study on human subjects. Participants performed a motor learning task with a rotation element just before going to sleep. Subjects also performed a control task without the rotation element prior to or after the experimental task. Although the tasks were almost indistinguishable, only the rotation task is understood to activate right parietal brain areas. Immediately after the rotation adaptation task, the researchers recorded participants' sleep for 2 hours. In addition to the expected general EEG sleep characteristics, it was discovered that after the experimental task (mental rotation), recordings showed a local increase in SWA within right parietal electrodes. Thus, the rotation adaptation task elicited a localized response in the sleep EEG that was not discovered in the control task. The researchers concluded that SWA can be selectively induced in restricted regions of the cerebral cortex (Huber, Ghilardi, Massimini, & Tononi, 2004).

It is difficult to find solid evidence for perhaps the most controversial statement of SHY, namely the causal role of SWA. Although Tononi and Cirelli validate that SWA and synaptic downscaling are closely related they have not produced direct evidence for a causal role. This has also been criticized by several researchers (Frank, 2012).

2.4 Critique of the SHY hypothesis

SHY has been criticized for being overly simplistic when interpreting molecular changes and their effects within the brain. In addition, several processes are highly regulated by the circadian system and could therefore account for some of the findings ascribed by SHY to sleep and waking. Furthermore, several prominent studies have reported results that conflict with certain assumptions and predictions of SHY.

2.4.1 Molecular change and synaptic effects

Results from several studies investigating gene expression and molecular changes within the sleep-wake cycle have been recapped to promote SHY. However, molecular changes do not inevitably reflect or accompany functional changes (Liu et al., 2010). In addition, relatively few molecules associated with plasticity have single synaptic effects. Molecules that are high in wake such as Arc and BDNF (Cirelli et al., 2004) have been interpreted as evidence for wake fostering LTP. Nevertheless, both Arc and BDNF do not solely facilitate LTP but additionally at times LTD and other similar processes (Frank, 2012). Vice versa molecules interpreted as evidence for synaptic downscaling during sleep are also required for some forms of LTP (Frank, 2012).

Noradrenaline levels, which Tononi and Cirelli have forwarded as one of the key candidate mechanisms for determining the direction of synaptic scaling, actually have complex effects on plasticity. For instance, noradrenaline can promote LTD depending on brain location and type of receptor (Frank, 2012). Thus, the presence of certain molecules alone does not necessarily predict the direction of synaptic change, neither whether there has occurred a functional change at all (Frank, 2012).

2.4.2 Brain temperature and corticosterone levels

Some of the strongest evidence in favor of SHY has derived from studies employing drosophila melanogaster. Drosophila is an ectothermic insect, and therefore does not have the ability to internally regulate its brain temperature. Ectothermic insects thus regulate brain temperature by modulating activity level or by changing to environments of suitable temperature (Stevenson, 1985). Several drosophila studies, including the study described by Bushey and colleagues have reported microstructural alterations during wake that accord with SHY. Fascinatingly, in drosophila, warm temperatures give rise to several of the same effects, such as increased axonal branching within the same neurons that were explored by Bushey and colleagues (Peng et al., 2007). Intriguingly, it has been documented that these temperature

effects are mediated by pathways which are shared with activity-dependent synaptic plasticity (Peng et al., 2007).

Numerous reports, including studies reviewed above, have concluded that in drosophila sleep reverses the structural alterations caused by wake. This reversal is considerably larger than effects reported by studies employing other organisms such as rodents (Frank, 2012). Actually, the only animals, which exhibit similar extensive structural downscaling during sleep-like states, are hibernators. During hibernation brain temperature swiftly declines and there is a massive retraction of dendrites and synapses (Ohe, Garner, Darian-Smith, & Heller, 2007). Thus, results from a large amount of studies employing drosophila melanogaster could in fact be caused by effects of brain temperature which is not in accord to the premises of SHY

In rodents the stress related hormone corticosterone is also regulated by the cycle of sleep and waking and levels are additionally increased during sleep deprivation. Moreover acute increases in corticosterone has been demonstrated to give rise to several structural alterations such as both frequency (Olijslagers et al., 2008) and the amplitude (Karst & Joëls, 2005) increases of mEPSCs in rat hippocampus. Numerous effects reported by rat studies and forwarded as support for SHY (as the study by Liu et al. where rats were sleep deprived) could in fact be caused by corticosterone increase (Frank, 2012).

2.4.3 Conflicting evidence

Although the exact mechanisms remain unclear, a dominating view within contemporary sleep research is that sleep promotes memory functions. SHY regards the positive influence of sleep on memory as an indirect result of net synaptic downscaling as it increases the signal to noise ratio (Tononi & Cirelli, 2006). Granted, several scientists support the notion that sleep fosters memory consolidation in a more narrow sense. This view does not stem from one well-defined model as is the case with SHY, but generally it is believed that neurons that are activated to encode information during wake are selectively reactivated during sleep (Born, Rasch, & Gais, 2006). During sleep the reactivated neurons will mainly fire within hippocampal ripples and neocortical sleep spindles (Grosmark, Mizuseki, Pastalkova, Diba, & Buzsaki, 2012). SHY avoids both the concept of consolidation and reactivation of memory traces, which is peculiar as large amounts of research supports this notion (Grosmark et al., 2012). Still, the two concepts are not necessarily mutually exclusive as a net decrease during sleep could occur in concert with reactivation of certain memory traces. Nevertheless, it is not intuitive how this would transpire as SHY predicts that the

"sleep-milieu" highly disfavors LTP.

By coupling an object location memory task with the odor of a rose, Rasch and colleagues elegantly manipulated memory reactivation during sleep (Rasch, Büchel, Gais, & Born, 2007). The task, which involved memorizing the location of certain cards, was performed in the evening. Subsequent sleep was monitored. During the first periods of SWS, the rose-odor was either re-presented or a vehicle was presented for the first time. Subjects were subsequently retested the following day. It was reported that compared to the vehicle presentation, memory of the card locations had greatly enhanced in the rose-odor re-exposure group. Results were not replicated when coupling the rose odor with a procedural tapping task, regardless of whether the re-exposure was presented within SWS or REM sleep. Moreover, re-presenting the odor to an additional group during the day did not enhance memory of card location significantly. To investigate their results further, fMRI was employed during post task sleep or during post task waking. As hypothesized, it was reported that re-exposure to the odor during SWS and wake activated left anterior and posterior parts of the hippocampus. Nevertheless, the effects were considerably weaker within the wake group. The researchers concluded that during slow wave sleep hippocampal networks were especially sensitive to stimuli that could prompt reactivation. Although these results do not exclude the possibility that other processes could be taking place within the cortex during SWS, the authors concluded in favor of the "consolidation model", stating that reactivations during SWS are causative factors for the consolidation of hippocampal memories (Rasch et al., 2007). It should be mentioned that although Rasch and colleagues reported odor-cued activation within hippocampus, they did not locate reactivation of memory traces per se. As fMRI was not employed during encoding, one cannot be certain that the memory traces that were active during the task were in fact the ones that were reactivated during nighttime.

SHY and similar models focus on the homeostatic abilities of SWS and do not attribute an explicit role to REM sleep (Grosmark et al., 2012). This is perhaps peculiar, as surprisingly few enquiries have been performed to exclude the homeostatic role of REM sleep (Grosmark et al., 2012). For instance several studies, (e. g. the article by Bushey et al. 2011 described above) discuss their findings in relation to sleep but also mostly in relation to SWS alone, even though the subjects have been deprived of both REM and SWS.

By examining spiking activity of rat hippocampal pyramidal cells and interneurons during sleep, Grosmark and colleagues investigated the specific roles of REM and NREM sleep, respectively (Grosmark et al., 2012). As rats lay down in sleeping position EEG was employed to separate REM from NREM sleep. The researchers discovered a significant

decrease in firing rates in both pyramidal cells and interneurons across the sleep period in total. These results are consistent with SHY. However, when analyzing the firing architecture within each NREM and REM period independently the researchers observed an *increase* in firing rate during SWS. This increase was counterbalanced by a substantial decrease in firing rate during REM sleep, which subsequently resulted in an overall net decrease. Additionally, Grosmark et al. discovered significant correlative evidence for theta wave involvement in synaptic downscaling (Grosmark et al., 2012). It should be noted that firing rate regulations within the cortex do not necessarily involve identical mechanisms as those operating within the hippocampus. Still, downscaling of cortical firing rates could in fact not be caused by SWA as stated by SHY but instead by intervening REM episodes, as observed within the hippocampus in this study (Grosmark et al., 2012).

2.5 Intention of current study

For obvious practical and ethical reasons no direct functional or microstructural evidence for SHY has derived from human studies. Sleep research employing human subjects has typically involved some type of sleep deprivation supplemented with cognitive tasks. Results from this line of research have emphasized immense cognitive deterioration caused by sleep deprivation (Durmer & Dinges, 2005). A number of human sleep deprivation studies have also employed fMRI to investigate corresponding deviations in neuronal activation, and both activity impairments and possible compensatory neuronal recruitment have been observed (Gujar, Yoo, Hu, & Walker, 2010).

Macrostructural consequences of sleep and sleep deprivation on the other hand have been sparsely explored within human participants. Recent research demonstrating that subtle structural brain differences can be detected in humans after only a few hours (Sagi et al., 2012) strongly suggests that inquiries into the macrostructural associations with sleep and sleep deprivation using MRI measures are feasible.

In addition, human sleep-related functional plasticity at the cellular level has been scarcely explored. VEP, a visual stimulus specific event-related potential (ERP) indexes synaptic transmission and likely aspects of functional plasticity within the visual cortex (Elvsåshagen et al., 2012). An averaged ERP consists of several positive and negative deflections that are called peaks or components. These peaks typically consist of a number to indicate the timing and a letter to indicate valence. The sequence of peaks following a stimulus is believed to reflect the sequence of neural processes beginning with early sensory processes and advancing through decision- and response-related processes (Lucka,

Woodmana, & Vogela, 2000). VEP collected during different phases in a sleep deprivation period could therefore provide novel insight into the neuronal and synaptic consequences of sleep and sleep deprivation. Moreover, as functional and structural plasticity are closely related it would be sensible to conduct studies measuring both manifestations of plasticity in conjunction.

As described, insect and rodent studies have several limitations such as possible confounds of brain temperature, stress and obvious caveats related to generalization to human neuroscience. Research employing human subjects is therefore needed. Thus, the main aim of the current study was to test specific predictions from SHY by investigating and characterizing structural and functional changes in cortical grey matter in human subjects as a function of sleep deprivation. We carried out a 24-hour sleep deprivation study using sensitive state-of-the art structural MRI and VEP assessments collected at three different time points; (1) in the morning after a good night's sleep, (2) in the evening after about 12 hours of wake, and (3) in the morning after 24 hours without sleep.

Based on predictions about synaptic effects of wakefulness our main hypothesis was that wakefulness would be associated with cortical thickening from morning to evening in concordance with a net increase in synapse potentiation. Furthermore, we anticipated that the synaptic potentiation would be accompanied by increased VEP amplitudes. As subjects were sleep deprived and synaptic downscaling could not occur, we further hypothesised that cortical thickening would continue or remain stable from evening until the following morning after sleep deprivation, as would the increases in VEP amplitudes.

3.0 Methods and materials

3.1 General overview and design

The current study was a longitudinal within subjects design sleep deprivation study coordinated from Oslo University Hospital, Rikshospitalet. The study was approved by the Regional Ethical Committee of South-Eastern Norway (REK Sør-Øst). A general overview of the study protocol is provided in Table 1. Subjects (see below for recruitment procedures etc.) arrived early in the morning, and extensive MR and VEP data were collected thereafter. Preceding the data collection, blood and saliva samples was attained. In total, the morning session lasted approximately 2-3 hours after which the subjects were free to leave hospital grounds but were firmly instructed not to fall asleep, have no intake of caffeine or energy drinks, or exercise. Subjects returned in the evening for an identical reassessment. Following

the evening data acquisition, subjects stayed overnight in an office room at the hospital. Subjects were instructed to play a videogame of their choice for a minimum of 3 hours. After completing the desired amount, most subjects continued to play videogames, while some watched a movie and spoke with the research assistant. As the deprivation period expanded and subjects became increasingly tired, struggling subjects were permitted to go for short walks within hospital grounds accompanied by the research assistant.

During the night, subjects also completed sheets of personal information, in addition to completing depressive and manic questionnaires. A research assistant monitored the participants at all times and no participant fell asleep during the night.

The final assessment was initiated precisely 24 hours after subjects had awoken the previous morning. Before commencing the last session, participants assessed their sleepiness by completing a questionnaire. The research assistant also completed a sleepiness scale on behalf of the subject. Total sleep deprivation period was approximately 27 hours as the final session commenced after 24 hours of sleep loss.

Morning 1 approx. 8 am	**Evening approx. 9 pm**	**Morning 2 approx. 8 am**
MR (resting state-fMRI, DTI and sMRI) 35 minutes	MR (resting state-fMRI, DTI and sMRI) 35 minutes	MR (resting state-fMRI, DTI and sMRI) 35 minutes
EEG/VEP 8 minutes	EEG/VEP 8 minutes	EEG/VEP 8 minutes
Saliva sample (cortisol)	Saliva sample (cortisol)	Saliva sample (cortisol)
Blood sample (hydration and health)	Blood sample (hydration and health)	Blood sample (hydration and health)
Went home (no sleeping, no training, no caffeine or energy drinks)	Stayed over night playing videogames and filling out questionnaires (no/little sugar intake)	Went home (experiment completed)

Table 1. Study protocol

3.2 Participants

21 male participants between the ages of 19-25 (Mean: 22.10 SD: 2.07) were recruited by student mail lists. After indicating their interest in the study current and prior medical history of the participant and family members was disclosed over the phone. Subjects were excluded if they had a history of depression, had any head injuries, were on medication, had a

high level of alcohol consumption or consumed any form of drugs other than nicotine. Subjects could not be severely addicted to nicotine, as they were not allowed any intake of it during the study. All participants were given a detailed oral description of the study in addition to a declaration form that was in compliance with the Helsinki Declaration. This form was read and signed before study onset. Subjects were also informed to arrive fasting with the exception of water before each session. Patients were insured through Pasientskadeforsikringsordningen and received NOK 1000,- for participating in the study.

3.3 MRI acquisition

Imaging was performed on a 3T Philips Achieva Scanner (Philips Healthcare, Eindhoven, the Netherlands) utilizing an 8-channel SENSE head coil at the Intervention Centre, Oslo University Hospital. The pulse sequence employed for volumetric analyses was a T1-weighted 3D turbo field echo (TFE) sequence (TR/TE = 8.4 ms/2.3 ms, FOV = 256 mm × 256 mm × 220 mm, 1 mm isotropic resolution, TA = 7 min 40 s). This sequence was run twice in order to increase signal-to-noise ratio.

Additionally resting-state fMRI and diffusion tensor imaging (DTI) data were collected, although this was beyond the scope of the current study. Participants were instructed to keep their eyes open at all times during the session and to focus on the most comfortable of 3 fixation crosses marked inside the scanner. It was specified that the participant could not under any circumstances fall asleep. During the fMRI acquisition patients were instructed to simply lay still and fixate on the cross. As the structural segments of the scan began patients listened to the radio channel of their choice in addition to performing a subtraction task. The subtraction task was difficult enough for subjects to stay alert and focused and they were also instructed to press a button as they reached certain numbers. For each button press a signal was given to the research assistants so that they could monitor their state of wakefulness. The scan session took approximately 35 minutes in total.

All patients orally confirmed that they had remained awake and performed the task as described within the first two scan sessions. During the third scan, as subjects struggled with the effects of sleep loss, several subjects reported that they had difficulties in staying awake. Still, as research assistants monitored their button pressing during the task, subjects could not have slept for more than a few seconds during the structural portions of the scan. A few subjects ceased pressing the button for a short period but instantly continued the task after the research assistant addressed them over the calling system. Granted, one subject ceased performing the task and did not respond to the research assistant. The completed T1 sequence

was examined for movement artifacts and as the T1 sequence was within good standard the rest of the session was aborted so that the following tests would not be affected by the subject having slept.

3.4 VEP acquisition

Continuous EEG was recorded by the use of 15 monopolar Ag/AgCl electrodes, according to the international 10–20 system. Impedances were maintained below 5 kΩ. Ground and reference electrodes were attached to the participants forehead. Eye movements were recorded by electrodes placed on the outside of each eye close to the temple in addition to placing electrodes above and under the right eye. The EEG was sampled at 250 Hz with an amplifier band-pass of 0.05–100 Hz.

VEP collection was completed at 3 different time points following MR acquisition. Participants were placed 97 cm from an LCD screen in binocular vision. They were instructed to continuously focus on a centrally presented filled red dot. The paradigm consisted of presenting either a grey background or a flickering checkerboard (2 reversals/s; check size = 0.5º). To avoid startling participants, the checkerboard was presented to subjects before the paradigm commenced for approximately 10 seconds. The checkerboard was presented by the use of E-Prime 1.1 (Psychology Software Tools, Inc., Sharpsburg, PA, USA).

During all three sessions, the VEP paradigm consisted of a flickering checkerboard for 2 short blocks (20 sec with 40 reversals) separated by periods of grey background of 1 or 5 minute duration. In total the experiment lasted 8 minutes.

Participants were instructed to blink as little as possible during the checkerboard phases and were allowed to listen to music during the data acquisition. In addition, all participants were monitored by web cam to ensure that they followed instructions and maintained awake throughout the session. If participants appeared to dose off, a research assistant would address them to keep them alert. Most participants completed all VEP acquisitions without significant trouble. One subject struggled excessively with staying awake during the last session and a research assistant had to engage in conversation numerous times.

3.5 Neuropsychology and psychometrics

For health purposes all participants were weighed before each session and blood pressure and pulse was measured during the evening. Furthermore, all participants filled out detailed questionnaires concerning general sleep patterns in addition to general demographic information such as work and living situation. After 24 hours of sleep deprivation each subject completed the Stanford sleepiness scale (Hoddes, Zarcone, Smythe, Phillips, &

Dement, 1973), which assesses degree of sleepiness. It consists of an 8-point scale ranging from highly alert and awake to asleep. Additionally the research assistant that had followed the subject completed a likert sleepiness scale on behalf of the subject ranging from 1-10 where 1 represented an alert and awake subject while 10 represented the subject being asleep.

Saliva and blood samples were collected at all three sessions. Blood samples were either taken by the research assistant or by a nurse, and saliva samples were collected by the research assistant by presenting the subjects with a piece of cotton which was placed in the mouth for 1 minute. Blood samples were collected to evaluate the health and hydration of individuals while saliva samples were collected to assess cortisol levels.

All participants underwent two clinical tests namely: The Montgomery-Asberg Depression Scale (MADRS) (Montgomery & Asberg, 1979) and Young Mania Rating Scale (YMRS) (Young, Biggs, Ziegler, & Meyer, 1978). Both tests include cognitive aspects such as subjective mindset and emotional state and are assumed sensitive to depressive and manic symptoms.

3.6 sMRI analysis

All datasets were processed and analyzed at the Neuroimaging Analysis Lab at KG Jebsen center for psychosis research, Oslo University Hospital. As mentioned fMRI and DTI analysis is beyond the scope of this study, and the following analysis is regarding sMRI. The analysis tool employed was FreeSurfer (version 5.1.0, http://surfer.nmr.mgh.harvard.edu) and the longitudinal analysis consisted of 3 steps typically referred to as Cross, Base and Long (Reuter, Schmansky, Rosas, & Fischl, 2012).

After sorting the data, we conducted the step called Cross where each time point was independently processed cross sectionally resulting in 63 datasets. The two repeated T1 sequences were extracted from the raw data and combined to increase the signal-to-noise ratio. Also, several preprocessing steps such as scull stripping, segmentation, intensity normalization and reconstruction of cortical surface was performed at this stage (Reuter et al., 2012). Cortical thickness at each point across the brain surface was automatically calculated as the shortest distance between pial surface and white matter (Fischl & Dale, 2000). All datasets were visually inspected and manually corrected if necessary.

The Base stage consisted of creating a template (also called base) by averaging the data from all three time points for each subject resulting in 21 datasets. Although there are several ways to create the template, we treated all time points equally to avoid possible asymmetries or biases (Reuter et al., 2012). The template was further visually inspected for

errors and corrected if necessary.

During Long, each time point was processed longitudinally. Directly copied information from the template and some information (not directly copied) from the cross-data were combined, and by the use of complex algorithms (Reuter et al., 2012) 63 datasets were created once more. At this stage manual editing was performed for the last time.

This three-steps approach has been shown to increase sensitivity to longitudinal differences (Reuter et al., 2012).The individual cortical thickness maps were subsequently resampled onto a common surface, smoothed with a full width at half maximum Gaussian kernel of 15 mm and submitted for higher-level analysis (Engvig et al., 2010).

3.7 VEP analysis

EEG analysis was conducted with EEGLAB, run by MATLAB 7.6.0. (MathWorks, Natick, MA, USA). The data was initially highpass filtered at 1 Hz. Subsequently epochs of 400 ms were extracted from the raw data. The epochs commenced at -50ms, which is 50 ms before the checkerboard was presented and therefore serves as a baseline. The epochs thus ended after 350 ms, and this action was performed for each checkerboard reversal. Epochs with amplitudes exceeding ±100 microvolts on any channels were rejected. This was implemented to remove strong artifacts such as eye blinks. Data was subsequently lowpass filtered at 30Hz to more accurately identify peak deflections. Subsequently data was baseline-corrected relative to the 50 ms pre-stimulus period and averaged to ERPs. ERPs were quantified by extracting values from the Oz channel (see below) resulting in C1 P1 and N1.

3.8. Statistical analysis of sMRI

Vertex-wise general linear models (GLMs) testing the differences in cortical thickness between: tp1 and tp2, tp1 and tp3, and tp2 and tp3 were performed. To further quantify the cortical alterations we divided the main clusters and extracted mean cortical thickness within each significant cluster across all time points. At this point in the analysis the data was thoroughly investigated both graphically and on the standard template to make initial judgments of the results.

In order to reduce the probability for type I errors, a statistical step was performed to correct for multiple comparisons. As Bonferroni correction can be overly conservative when employed on MRI data (Hagler, Saygin, & Sereno, 2006) we performed a correction based on cluster size inference by means of Z Monte Carlo simulations. By the use of FreeSurfer, clusters were tested against an empirical (non-parametric) null distribution of maximum cluster size. Initial cluster-forming threshold employed was $p < 0.05$ (Hagler et al., 2006).

Millimeter difference between the first and the two latter scans were transformed into percent difference in order to facilitate plotting of group-averaged data. Furthermore, to assess a possible relationship between percent thickness change within each cluster and degree of sleepiness, bivariate Pearson's correlation coefficients (*r*) were calculated. To correct for multiple analyses on the same dataset the alpha level was Bonferroni corrected with a factor of 14 (corresponding to the number of tests) resulting in a corrected alpha level of 0.004. Additionally, post-hoc analyses were run in order to investigate possible relationships between structural and functional alterations using Pearson's correlation analysis. The corrected alpha level was employed.

3.9 Statistical analysis of VEP

In order to quantify amplitude alterations of C1, P1, and N1 data was transformed into absolute values. To assess whether possible alterations were statistically significant, paired samples *t* tests with an alpha level of 0.05 were performed in SPSS on relevant VEP measures (C1, P1 and N1 amplitudes). To correct for multiple analyses on the same dataset, the alpha level was Bonferroni corrected (0.05/9) resulting in a new alpha of 0.006. To assess the size and direction of a possible relationship between absolute values within each VEP and degree of sleepiness, bivariate Pearson's correlation coefficients were calculated. The Bonferroni corrected alpha level was employed.

3.10 General- and statistical analysis of neuropsychological- and psychometrics tests

All blood samples were analyzed at Oslo University Hospital, Rikshospitalet and pulse and blood pressure was evaluated on the spot to assess subject health. Hydration effects within the blood and cortisol effects within saliva could not be analyzed in time of submission. MADRS, YMRS, SSS, and the likert sleepiness scale were all analysed in SPSS for computation of summary statistics and general descriptives. General sleep pattern the last year and demographic information were not analysed as it is beyond the scope of this study.

4.0 Results

4.1. Psychometrics

The YMRS test ranges from the minimum score of 0 to the maximum score of 60. Within our study 7 was employed as a cut off, thus scoring above 7 would imply that the subject could be manic at the time. The YMRS scores ranged from 0 to 4 (M= 0.90, SD= 1.35). Thus we concluded that none of our subjects were manic at the time of data acquisition.

MADRS ranges from the minimum score of 0 to the maximum score of 60. The

MADRS scores ranged from 0 to 22 (M= 3.52, *SD*= 4.91). One subject scored 22 indicating a possible depression. As several theories have suggested that plasticity might be affected by depression (Duman, 2002), this dataset was inspected for deviations compared to the rest of the group. No deviations were found, and the dataset was not excluded from the analyses.

The SSS scale ranges from the minimum score 1 to the maximum score of 8 (admittedly indicating asleep). Scores ranged from 2 to 7 (*M*=4.5, *SD*= 1.14). The likert sleepiness scale completed by the research assistant ranged from 1 to 10. Subjects scored within the range of 4 and 9 (*M*= 5.32, *SD*= 1.52). It should be mentioned that correlations between subjective and assistant scores were mildly negative. Also, assistant scores were not collected for 2 of the subjects thus only 19 datasets were analyzed.

4.2. sMRI

Figure 2 displays the results from the vertex-wise GLM analysis testing the differences in cortical thickness between each time point. Five clusters of significant ($p<0.05$, corrected, right panel) grey matter variation were identified, including four clusters reflecting significant differences between time point (tp) 1 and 2 and one cluster reflecting differences between tp 1 and 3. The four former clusters comprised frontal and temporal regions bilaterally within superior frontal cortex, inferior temporal cortex and additionally within middle frontal cortex only within the left hemisphere. The latter cluster was highly overlapping with the left frontal cluster from the first contrast.

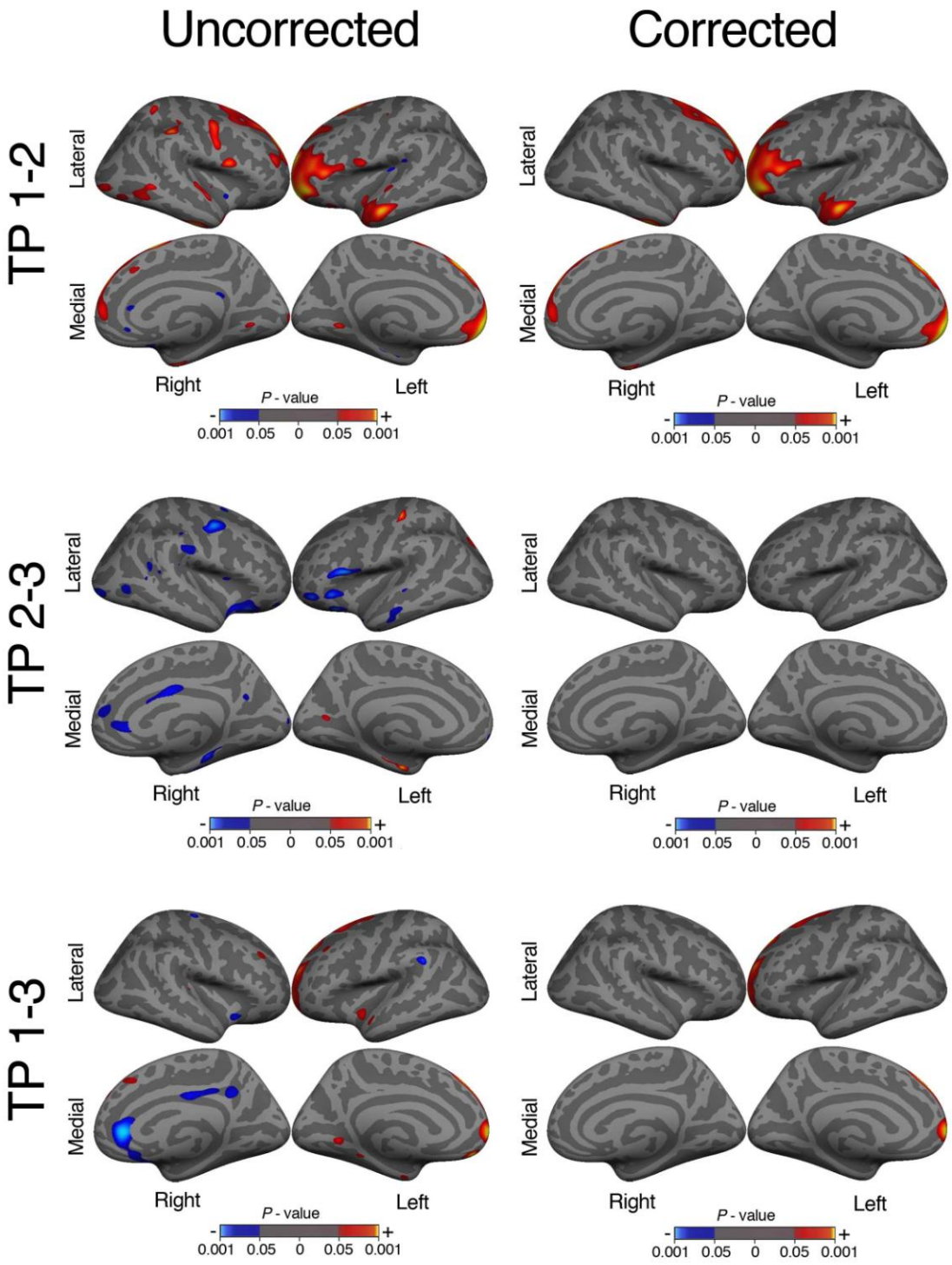

Figure 2. Uncorrected ($p < .05$, left panel) and corrected ($p < .05$, right panel) GLM analysis results. Five significant clusters are depicted. Blue color represents cortical thinning, while red color represents cortical thickening between the different assessments.

Figure 3 displays the localization and the percent difference in cortical thickness between baseline and the two later assessments in the various clusters. There is an increase in cortical thickness from the first morning and to the following evening ranging from 1 to about 2.5% in the various clusters. Cluster 1 (left prefrontal cortex) has the largest alteration while cluster 4 (right temporal cortex) has the smallest. From evening and to the following morning, there seem to be a trend toward a slight decrease in thickness but not enough to reach baseline scan measurements or significane. It should be specified that we know nothing of the continuous cortical development between the 3 scanning sessions. Although straight lines between scanning time points are depicted in the figure, which might give the illusion of a smooth increase or decrease, it is not known how these results transpire.

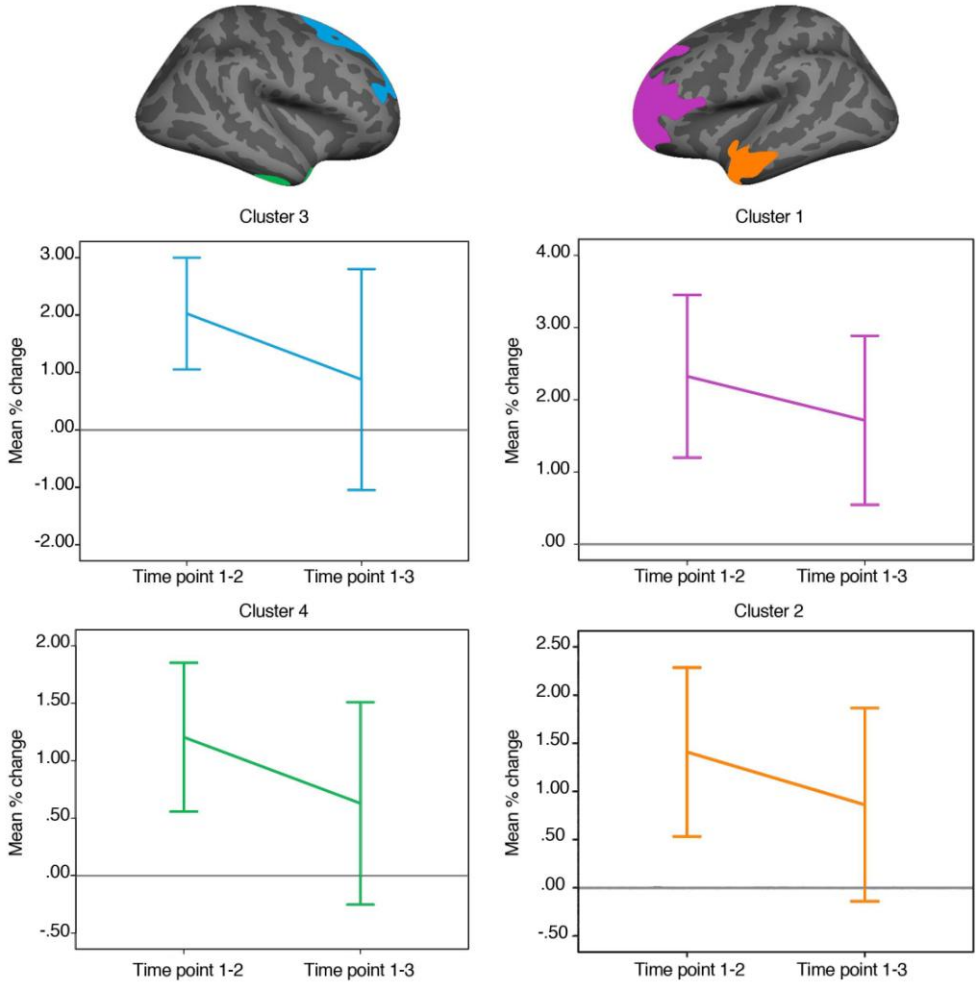

Figure 3. Upper panel: Localizations of significant clusters from the GLM testing the difference in cortical thickness between tp1 and tp2. Lower panels: Percent difference in cortical thickness between tp 2 and 3 relative to baseline. Error bars depict 95 % confidence interval. The grey horizontal line at zero represents the first scan, which can be understood as a baseline

Within cluster 1, thickness changes between tp 1 and 2 were on average -0.06mm (t_{diff} (20)= -4.26). The difference between tp 1 and 3 were on average -0.04mm (t_{diff}(20)= -3.07). Within cluster 2, thickness changes between tp 1 and 2 were on average -0.05mm (t_{diff}(20)= -3.41). Within cluster 3, thickness changes between tp 1 and 2 were on average -0.05mm (t_{diff}(20)= -4.30). Within cluster 4, thickness changes between tp 1 and 2 were on average -0.04mm (t_{diff}(20)= -3.83). It should be mentioned that negative values are in fact increases as tp 2 and 3 were subtracted from tp 1.

	Tp1		**Tp 2**		**Tp 3**		**Tp 1-2**		**Tp 1-3**		**Tp 2-3**	
	M	SD	M	SD	M	SD	t_{diff}	p	t_{diff}	p	t_{diff}	p
Cluster 1	2.60	0.13	2.66	0.13	2.65	0.16	-4.26	0.001	-3.07	0.006	0.74	0.469
Cluster 2	3.26	0.18	3.33	0.18	3.29	0.20	-3.41	0.003	-1.87	0.007	1.50	0.149
Cluster 3	2.64	0.08	2.69	2.69	2.66	0.14	-4.30	0.001	-0.96	0.351	1.10	0.285
Cluster 4	3.41	0.17	3.45	0. 17	3.43	0.17	-3.83	0.001	-1.47	0.156	1.71	0.103

Table 2. Descriptives and accompanying *t* values and significance levels for each cluster. Note that "1-2": tp2 subtracted from tp1 thus negative values are increases.

4.2.1 Correlations between cortical thickness and sleepiness

All Pearson's correlation coefficients (Table 3) between all clusters and both measurements of sleepiness were non-significant. These results indicate no relationships between feeling or acting sleepy and the structural alterations reported in our study.

		Subjective score		Assistant score	
		R	Sig. (2-tailed)	R	Sig. (2-tailed)
Cluster 1	Tp 1-2	0.15	0.504	0.04	0.871
	Tp 1-3	-0.27	0.243	-0.00	0.997
Cluster 2	Tp 1-2	0.09	0.691	0.05	0.836
	Tp 1-3	0.17	0.451	0.15	0.531
Cluster 3	Tp 1-2	0.12	0.621	0.12	0.631
	Tp 1-3	-0.19	0.402	-0.08	0.752
Cluster 4	Tp 1-2	0.05	0.833	0.15	0.545
	Tp 1-3	-0.07	0.765	-0.08	0.758

Table 3. Depiction of Pearson's correlation coefficients and significance level between sleepiness data scored by subject and by assistant and each cluster. R: Person's correlation coefficient.

4.3. VEP amplitudes

Amplitudes were located for: C1 (most negative value between 110 and 450ms), P1 (most positive value between 90 and 150ms) and N1 (most negative value between 130 and 190 ms). As presented in Figure 5 there is a trend toward variation in amplitude for all VEP components across all time points. As with the prior figure it should be specified that we know nothing of the transpiration of the amplitude variation between data acquisition time points.

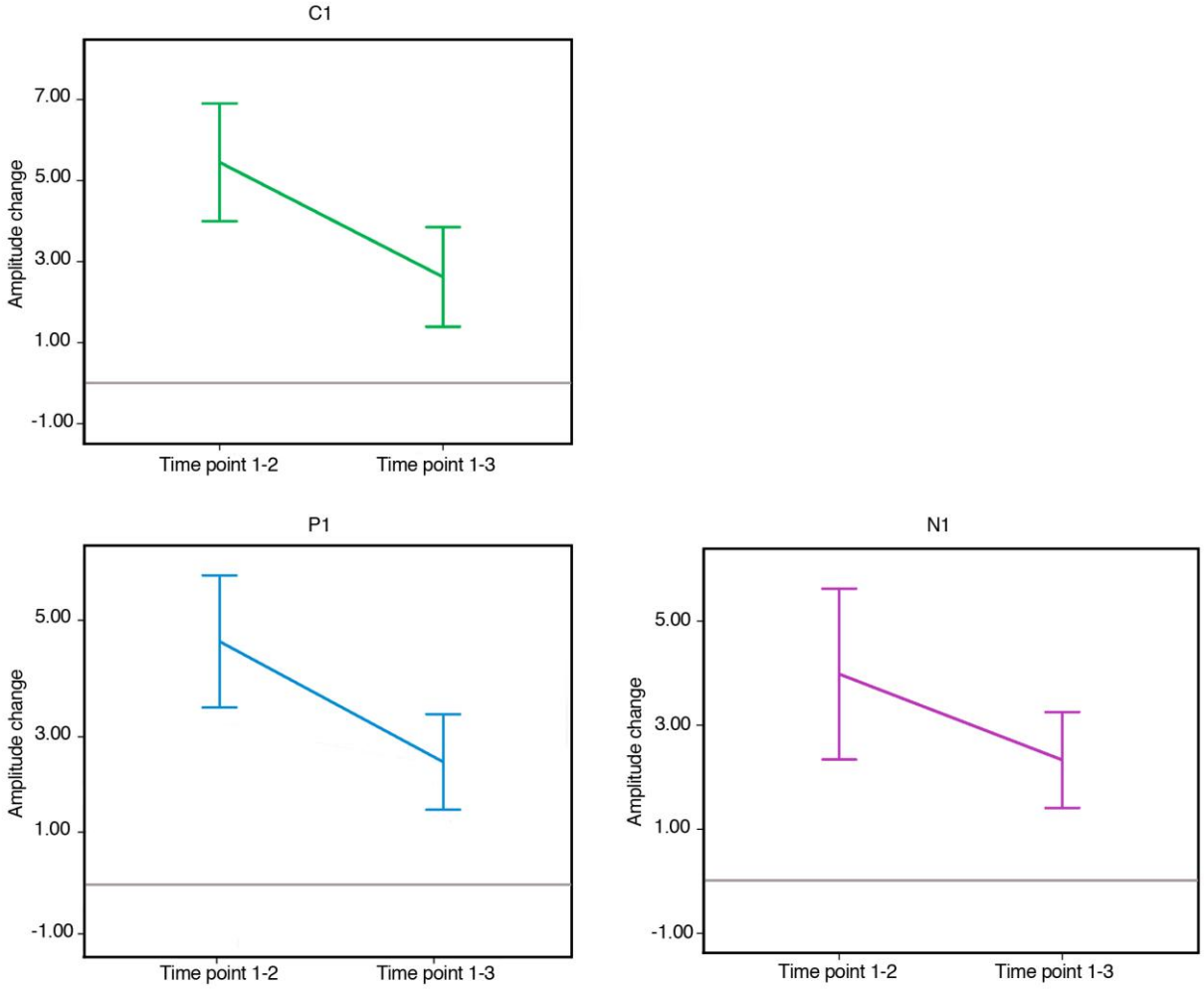

Figure 5. C1, P1 and N1 amplitude change at all three time points by the use of absolute values. Confidence interval of 95% is depicted in the graph and the grey horizontal bar illustrates the baseline or first data acquisition. C1, P1 and N1 increase during the day becoming more positive and negative respectively. During evening and to the following morning all amplitudes return somewhat to their original state but not as much as during baseline measurements. All trends were statistically non-significant)

Within C1, amplitude changes between tp 1 and tp 2 were on average -0.59µV. The difference in amplitude between tp 1 and tp 3 was on average -1,51µV and -0.92µV between tp 2 and 3. Within P1, amplitude changes between tp 1 and tp 2 was on average -1.13µV. The difference in amplitude between tp 1 and tp 3 was on average -1.27 µV and -0.14µV between tp 2 and 3. Within N1, amplitude changes between tp 1 and tp 2 were on average 0.03 µV. The difference between tp 1 and tp 3 was on average -0.18µV and -0.20µV between tp 2 and 3. Neither of the microvolt changes was statistically significant (see table 4). The results of the *t* tests thus indicate that although there were trends depicted in Figure 5. none of these

alterations actually reached statistical significance.

	Tp1		**Tp 2**		**Tp 3**		**Tp 1-2**		**Tp 1-3**		**Tp 2-3**	
	M	SD	M	SD	M	SD	t_{diff}	p	t_{diff}	p	t_{diff}	p
C1	-6.35	4.21	-5.76	3.90	-4.84	4.36	-0.42	0.678	-1.99	0.061	-0.65	0.525
P1	4.22	5.51	5.35	4.62	5.48	4.97	-0.95	0.353	-2.00	0.059	-0.12	0.908
N1	-4.98	4.19	-5.00	3.71	-4.80	3.43	0.02	0.982	-0.26	0.800	-0.19	0.852

Table 4. Descriptives and accompanying *t* value and significance level for each VEP. Note that "1-2": tp2 subtracted from tp1 thus negative values are increases.

4.3.1 Correlations between VEP and sleepiness

All Pearson's correlation coefficients (Table 5) between VEP change and both measurements of sleepiness were non-significant. These results indicate no relationships between feeling or acting sleepy and the functional alterations reported in our study.

		Subjective score		**Assistant score**	
		R	Sig. (2-tailed)	R	Sig. (2-tailed)
C1	Tp 1-2	0.19	0.404	0.47	0.041
	Tp 1-3	0.14	0.557	-0.17	0.501
P1	Tp 1-2	-0.32	0.155	-0.22	0.366
	Tp 1-3	-0.01	0.955	-0.26	0.292
N1	Tp 1-2	-0.03	0.908	0.04	0.873
	Tp 1-3	0.09	0.691	-0.13	0.591

Table 5. Depiction of Pearson's correlation coefficients and significance level between sleepiness data scored by assistant and subject and VEP components. R: Person's correlation coefficient.

4.4 Correlations between structural and functional measures

All correlation coefficients (see table 6) between all clusters and all VEP components were statistically non-significant. These results indicate that there is no relationship between functional and structural alterations reported in this study.

	Cluster1 tp 1-2	Cluster1 tp 1-3	Cluster2 tp 1-2	Cluster2 tp 1-3	Cluster3 tp 1-2	Cluster3 tp 1-3	Cluster4 tp 1-2	Cluster4 tp 1-3
C1 tp 1-2	-0.12	-0.38	-0.19	-0.21	-0.11	-0.20	-0.26	-0.34
C1tp 1-3	-0.13	-0.19	-0.15	-0.19	0.06	-0.08	0.12	-0.23
P1 tp 1-2	0.07	-0.22	0.05	-0.01	0.08	0.13	0.33	0.32
P1tp 1-3	-0.07	-0.17	-0.09	0.16	-0.38	-0.22	0.11	0.11
N1 tp1-2	0.35	0.41	0.39	0.19	0.32	0.01	0.15	-0.15
N1 tp1-3	-0.11	-0.13	-0.14	0.10	-0.27	0.10	-0.08	-0.02

Table 6. Person's correlation coefficients between structural and functional data.

5.0 Discussion

Third mate Gregory Cousins was simply performing a standard turn when it all went terribly wrong and he for some reason struck a reef (Adasiak et al., 1990). Owing to large amounts of research, one today recognizes that the failed turn by a fatigued boatman was probably caused by the detrimental effects sleep deprivation has on cognitive abilities. Although the knowledge of sleep has broadened considerably since the time of Aristotle, a proper understanding of sleep function is still lacking. Nevertheless, the synaptic homeostasis hypothesis, and its propositions on sleep function have grown to be one of several dominant theories (Frank, 2012) within the field. As certain aspects of the hypothesis have been challenged, research employing new methods and human subjects could inform the current debate. In line with our hypotheses, by employing highly sensitive longitudinal measures of cortical thickness we have reported that wake foster measurable macrostructural increases in grey matter, presented as 4 clusters: within superior frontal cortex bilaterally, left middle frontal cortex and inferior temporal cortex bilaterally. This increase was not significantly alternated during a period of sleep deprivation. The implication of these results for the debate on sleep function will be discussed in the following.

5.1 Macrostructural increases in grey matter

As initially hypothesized we reported in the current study that cortical thickness increased from morning to evening, resulting in 4 significant bilateral clusters. The left frontal cluster was spatially the largest and additionally showed the largest differences in terms of percentiles. If these results genuinely mirror alterations within the brain the possibilities are

either intracellular or extracellular. Thus structural alterations do not necessarily mirror changes within a cell but could in fact be caused by alterations of the space between cells. A possible extracellular candidate could be water. It has been reported that subjects restricted to low levels of water for 2 days had significant decreases in grey matter volume (Streitbürger et al., 2012). Indeed, there might be a hydration effect in our study as one is typically less hydrated early in the morning compared to evenings as one has not been drinking while asleep (Armstrong et al., 2010). Although our subjects were allowed to drink as they pleased there is still a possibility for increased hydration after a full day of water intake. Unfortunately, due to methodological constraints, the anatomical loci of the hydration effects reported by previous studies including the left caudate nucleus and right-cerebellar posterior lobe cannot be replicated using the current estimates of cortical thickness. However, as subjects drank all through the night there should be no trend toward a decrease in cortical thickness between time point 2 and 3 if our results are solely based on hydration effects. Moreover it is not intuitive why there would be lateral effects if they were caused by variability in hydration.

It is also important to discuss possible scanner artefacts such as scanner temperature. In the current study, when scans were performed in the morning our subjects were the first to enter the scanner that day. In the evening on the other hand our subjects were the last individuals to get scanned after the scanner had consequently been employed for a full day. Thus, it is possible that the scanner was cooler in the morning compared to the evening consequently causing the reported results (be it expressed in an extracellular or intracellular fashion). Still, in addition to lateral effects there is a significant difference in cluster 1 between the first and the second morning (with uncorrected alpha level) in which the scanner should be within approximately equal temperature. It is not intuitive why these effects would transpire if they were solely caused by scanner artefacts related to time of scanning etc.

If the results reported are intracellular a possible underlying mechanism could be features connected to LTP. As grey matter alterations have occurred after approximately 12 hours, neurogenesis or any other forms of L-LTP cannot be the underlying cause. There is evidence on the other hand (Bliss & Cooke, 2011) for E-LTP occurring after only a few hours. Thus our results might possibly be connected to some of the earliest manifestations of naturally occurring plasticity. Although one can only speculate as to what manifestations have been detected, the results are still in concordance with the premises of SHY. As described earlier functional synaptic strength (which supposedly expand across the day), is most likely initiated by structural alterations (Castren & Hen, in press). Hence, it is logical that highly potentiated synapses are somehow larger than less potentiated synapses. Accordingly, the

current study might have detected the structural "instigator" of the functional daytime increase forwarded by SHY and reported from several studies.

There were no significant differences in cortical thickness between time point 2 and 3. Although somewhat difficult to assess, we hypothesized that cortical thickness should either expand or remain stabile during the period of sleep deprivation. This with the logic that synapses would not be able to become downscaled during continued wakefulness. As there were no significant alterations our initial hypothesis of stabilisation was confirmed. Why there was not a continuing grey matter increase could be because synapses had already reached their maximum potentiation level. Indeed our results fit well with other articles reporting that extending waking period beyond its natural physiological duration did not result in further structural increases (Maret, Faraguna, Nelson, Cirelli, & Tononi, 2011). Admittedly, there was a non-significant trend toward a decrease in cortical thickness during extended sleep deprivation, and one cannot exclude the possibility of this trend being significant with a larger N. Although speculative, such a decrease could be related to effects of stress or downscaling during micro sleep episodes, both of which we could not control for in the present study.

Why are the structural alterations reported mostly within frontal areas? Following the premises of SHY one would possibly expect a non-specific increase in synapses across cortex. Nevertheless, synaptic potentiation could according to SHY transpire locally (Tononi & Cirelli, 2006) and be positively modulated by usage. It is therefore a possibility that frontal areas were frequently accessed during a day of school and studying, as our sample mostly comprised of students.

5.2 No relationship between sleepiness or functional data and structural data

We found no effects of sleepiness on the structural results reported and this is perhaps counterintuitive within the logic of SHY. Would not synapses of maximum weight result in a dire sleep need? And are not increases in subjective and behavioural sleepiness connected to an elevated physical drive for sleep? Not necessarily. The subjective feeling of sleepiness is not always proportionate to actual sleep need. Sleep regulation is partly controlled by circadian rhythm, a biological clock that regulates the sleep wake cycle (Borbély & Tononi, 1998). The circadian rhythm can be affected by "external signs of morning" such as temperature and light (Honma, Honma, Kohsaka, & Fukuda, 1992). Consequently, sleep deprived individuals might feel sleepier around 5 am. when it is colder and darker compared to at 7am. when it is warmer and lighter. Moreover, as our two assessments of sleepiness correlated negatively, alternative sleep measurements such as EEG could possibly have given

different results.

Although there are strong connections between structural and functional plasticity we found no relationship between these aspects in our current study. This could be since we are measuring possible alterations within two different brain areas and that these effects are not mediated across the brain. Besides, following our earlier incentive of what the structural alterations supposedly depicted, it is only logical that we did not find any functional alterations in primary visual cortex as we found no structural alterations within this area. As we did find significant structural alterations within frontal areas on the other hand, it is more feasible to expect a possible functional alteration within this area although this would only be speculation.

5.3 Limitations of current study and future research

If the structural daytime alterations reported in the current study are in fact intracellular they might nevertheless be evidence *against* the SHY hypothesis. Stress related hormones called Glucocorticoids are highly regulated by the circadian system (Mongrain et al., 2010). Glucocorticoids reach their extreme during wake, low point during sleep and levels are additionally elevated during sleep deprivation (Mongrain et al., 2010). Stress is hence a problematic confound for several of the premises of SHY and additionally for sleep deprivation studies in general. Intriguingly, glucocorticoids can effect synapses in a way that closely resembles the alterations hypothesized by SHY (Frank, 2012). Corticosterone the rodent equivalent of cortisol has as previously described profound effects on synaptic efficacy and plasticity-related molecules (Frank, 2012). In prolonged waking corticosterone has been reported to both enhance neuronal excitability and promote LTP (Joëls, Krugers, & Karst, 2008). Although less is known about the effects of cortisol, one cannot exclude the possibility that our daytime results were not caused by wake per se but instead by cortisol levels that are regulated by wake. Unfortunately, cortisol levels could not be analysed in due time for the present thesis, but future research should get a better understanding of the effects of cortisol and correct or control for these effects as best possible. Nevertheless it should be mentioned that although cortisol levels increase during sleep deprivation we did not in fact find any significant alterations within this period.

Within the current study we had little control over certain aspects of subject sleep. Subjects were instructed to get a good night sleep prior to the first assessment. We employed the first scan as a baseline assuming that subjects had followed our instructions. Still, as they were not monitored as they slept, we have no knowledge of their sleep quality or if they even

slept at all. Additionally, the first scan was conducted early in the morning, and certain subjects thus had to wake up as early as 6 am, possibly affecting their normal sleep pattern. Moreover as subjects were free to leave after the first data acquisition, we had little control over what subjects did during the day. One cannot exclude the possibility that subjects drank coffee, exercised or even slept during the day. Micro sleep episodes during sleep deprivation or moments of sleep during resting state fMRI were additionally not controlled for. Thus future research should employ higher control over subject sleep both the night before and within the 24 hour study. Eye tracking could additionally be employed during MR acquisitions and EEG could be employed during the sleep deprivation period to study both micro sleep episodes and sleepiness as this can be detected by EEG (Mardi, Ashtiani, & Mikaili, 2011Mardi, Ashtiani, & Mikaili, 2011).

In our study all VEP trends were ultimately statistically non-significant thus challenging our initial hypothesis. The trend of an amplitude increase during the day would have been in concordance with the SHY hypothesis, and one could speculate if a larger N would have resulted in significant effects. Moreover our subject group consisted of only men and within fairly young ages (19-25). Thus, it might be hard to generalize our results to other groups. Additionally we have no information on how the daytime structural alterations transpired within the current study as we only had one scan during the morning and one during the evening. Furthermore, although the within-subject longitudinal design provide a sensible approach for studying effects of sleep deprivation, we could not compare our results to a group which was not sleep deprived. Thus future research should employ larger subject groups, including both men and women and employ additional scans during daytime to clarify how the temporal and directional effects of cortical variation transpire. Moreover, future research should have a group of sleeping controls to increase our understanding of cortical variation during sleep and sleep deprivation.

As our subjects were deprived of all sleep, and as few explorations have been performed earlier, if would be profitable if impending research explored the relationship between SWS and REM more closely and employed selective deprivation methods. In addition as it is unclear whether neuronal activity varies depending on brain region during sleep this should be investigated further. Future research should additionally increase the understanding of hydration effects and cortisol level, on cortical thickness.

6.0 Conclusion

Our initial hypotheses were validated as we found 4 significant bilateral clusters of grey matter increase during a day of wake. This increase was not significantly reduced during sleep deprivation. After careful consideration and discussion it is concluded that these results can be employed as additional evidence for the SHY hypothesis. Nevertheless it would be rewarding if future research got a better understanding of the relationship between REM and SWS and effects of brain temperature as these are weaknesses within the synaptic homeostasis hypothesis. Additionally impending research should get a better understanding of hydration- and cortisol level effects, as these are possible confounds within the current study.

References

Adasiak, Allan, Ricbter, Ceceile Kay, & Spivey, Pete. (1990). SPILL The Wreck of the Exxon Valdez. In S. Lindbeck & J. Brogan (Eds.), (pp. 1-211). Alaska: Alaska Oil Spill Commission.

Almer, G., Hainfellner, J. A., Brucke, T., Jellinger, K., Kleinert, R., Bayer, G., . . . Budka, H. (1999). Fatal familial insomnia: a new Austrian family. *Brain, 122*, 5-16.

Andrés, Isabel de, Garzón, Miguel, & Reinoso-Suárez, Fernando. (2011). Functional Anatomy of Non-REM Sleep. *Frontiers in neurology, 2*(70), 1-14. doi: 10.3389/fneur.2011.00070

Armstrong, L. E., Pumerantz, A. C., Fiala, K. A., Roti, M. W., Kavouras, S. A., Casa, D. J., & Maresh, C. M. (2010). Human hydration indices: acute and longitudinal reference values. *International journal of sport nutrition and exercise metabolism, 20*(2), 145-153.

Azar, Beth. (2006). Wild findings on animal sleep: Do the similarities of sleep across the animal world indicate a universal function or just convergent evolution? *37*(1), 54. http://www.apa.org/monitor/jan06/wild.aspx

Benington, J. H., & Frank, M. G. (2003). Cellular and molecular connections between sleep and synaptic plasticity. *Progress in neurobiology, 69*(2). doi: 10.1016/S0301-0082(03)00018-2

Bergh, Bea R. H. van den, & Mulder, Eduard J. H. (2012). Fetal sleep organization: A biological precursor of self-regulation in childhood and adolescence? *Biological Psychology, 89*(3), 584-590. doi: 10.1016/j.biopsycho.2012.01.003

Blake, H., & Gerard, R. W. (1937). Brain potentials during sleep. *American Journal of Physiology, 119*(4), 692-703.

Bliss, T. V. P., & Cooke, I. S. F. (2011). Long-term potentiation and long-term depression: a clinical perspective. *CLINICS, 66*(S1), 3-17. doi: 10.1590/S1807-59322011001300002

Bliss, T. V. P., & Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *THe journal of physiology, 232*(2), 331-356.

Borbély, Alexander A., & Tononi, Giulio. (1998). The quest for the essence of sleep. *Daedalus, 127*(2), 167-196.

Born, Jan, Rasch, Bjørn, & Gais, Steffen. (2006). Sleep to remember. *Neuroscientist, 12*(5), 410-424. doi: 10.1177/1073858406292647

Braun, A. R., Balkin, T. J., Wesensten, N. J., Carson, R. E., Varga, M., Baldwin, P., . . . Herscovitch, P. (1997). Regional cerebral blood flow throughout the sleep-wake cycle. An H2(15)O PET study. *Brain, 120*(7), 1173-1197.

Bushey, Daniel, Tononi, Giulio, & Cirelli, Chiara. (2011). Sleep and Synaptic Homeostasis: Structural Evidence in Drosophila. *Science, 332*(6037), 1576-1581. doi: 10.1126/science.1202839

Carskadon, M. A., & Dement, W. C. (2011). Normal Human Sleep : An Overview. In M. H. Kryger, T. Roth & W. C. Dement (Eds.), *Principles and practice of sleep medicine* (5th ed., pp. 16-26). St. Louis: Elsevier Saunders.

Castren, Eero, & Hen, Rene. (in press). Neuronal plasticity and antidepressant actions. *Trends in Neurosciences*. doi: 10.1016/j.tins.2012.12.010,

Cirelli, Chiara, Gutierrez, Christina M., & Tononi, Giulio. (2004). Extensive and Divergent Effects of Sleep and Wakefulness on Brain Gene Expression. *Neuron, 41*(1), 35-43.

Derrick, Martha L. Escobar and Brian. (2007). Long-Term Potentiation and Depression as Putative Mechanisms for Memory Formation. In B.-R. F & B. R. F. C. Press; (Eds.), Neural Plasticity and Memory: From Genes to Brain Imaging. Boca Ration: CRC Press. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK3912/.

Draganski, B., Gaser, C., Busch, B. V., Schuierer, G., Bogdahn, U., & May, A. (2004). Neuroplasticity: Changes in grey matter induced by training. *Nature, 427*(6972), 311-312. doi: 10.1038/427311a

Duman, R. S. (2002). Pathophysiology of depression: the concept of synaptic plasticity. *European Psychiatry, 17*(3), 306-310. doi: 10.1016/S0924-9338(02)00654-5

Durmer, Jeffrey S., & Dinges, David F. (2005). Neurocognitive Consequences of Sleep Deprivation. *Seminars In Neurology, 25*(1), 117-129. doi: 10.1055/s-2005-867080

Elvsåshagen, T., Moberget, T., Bøen, E., Boye, B., Englin, N. O., Pedersen, P. Ø., . . . Andersson, S. (2012). Evidence for impaired neocortical synaptic plasticity in bipolar II disorder. *Biological Psychiatry, 71*(1), 68-74. doi: 10.1016/j.biopsych.2011.09.026

Engvig, A., Fjell, A. M., Westlye, L. T., Moberget, T., Sundseth, Ø., Larsen, V. A., & Walhovd, K. B. (2010). Effects of memory training on cortical thickness in the elderly. *Neuroimage, 52*(4), 1667-1676. doi: 10.1016/j.neuroimage.2010.05.041

Eriksson, Peter S., Perfilieva, Ekaterina, Björk-Eriksson, Thomas, Alborn, Ann-Marie, Nordborg, Claes, Peterson, Daniel A., & Gage4, Fred H. (1998). Neurogenesis in the adult human hippocampus. *Nature medicine, 4*, 1313-1317. doi: 10.1038/3305

Feinberg, I., & Floyd, T. C. (1979). Systematic Trends Across the Night in Human Sleep Cycles. *Psychophysiology, 16*(3), 283–291.

Fischl, B., & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *PNAS, 97*(20), 11050-11055.

Frank, Marcos Gabriel. (2012). Erasing synapses in sleep: is it time to be SHY? *Neural Plasticity, 2012*(264378), 1-15. doi: 10.1155/2012/264378

Gilestro, Giorgio F., Tononi, Giulio, & Cirelli, Chiara. (2009). Widespread Changes in Synaptic Markers as a Function of Sleep and Wakefulness in Drosophila. *Science, 324*(109), 109-112. doi: 10.1126/science.1166673

Grosmark, Andres D., Mizuseki, Kenji, Pastalkova, Eva, Diba, Kamran, & Buzsaki, Gyorgy. (2012). REM Sleep Reorganizes Hippocampal Excitability. *Neuron, 75*(6), 1001-1007. doi: 10.1016/j.neuron.2012.08.015

Gujar, Ninad, Yoo, Seung-Schik, Hu, Peter, & Walker, Matthew P. (2010). The Unrested Resting Brain: Sleep Deprivation Alters Activity within the Default-mode Network. *Journal of Cognitive Neuroscience, 22*(8), 1637–1648. doi: 10.1162/jocn.2009.21331

Hagler, Donald J., Saygin, Ayse Pinar, & Sereno, Martin I. (2006). Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *Neuroimage, 33*(4), 1093-1103. doi: 10.1016/j.neuroimage.2006.07.036

Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., & Dement, W. C. (1973). Quantification of Sleepiness: A New Approach. *Psychophysiology, 10*(4), 431-436. doi: 10.1111/j.1469-8986.1973.tb00801.x

Honma, K., Honma, S., Kohsaka, M., & Fukuda, N. (1992). Seasonal variation in the human circadian rhythm: dissociation between sleep and temperature rhythm. *American Journal of Physiology, 262*(5), 885-891.

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

Jenkins, John G., & Dallenbach, Karl M. (1924). Obliviscence during sleep and waking. *The American Journal of Psychology, 35*(4), 605-612.

Joëls, Marian, Krugers, Harm, & Karst, Henk. (2008). Stress-induced changes in hippocampal function. *Progress in brain research, 167*, 3-15.

Karst, H., & Joëls, M. (2005). Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells. *Journal of Neurophysiology, 94*(5), 3479-3486.

Knott, Graham W., Quairiaux, Charles, Genoud, Christel, & Welker, Egbert. (2002). Formation of Dendritic Spines with GABAergic Synapses Induced by Whisker Stimulation in Adult Mice. *Neuron, 34*(2), 265-273. doi: 10.1016/S0896-6273(02)00663-3

Lim, Julian, & Dinges, David F. (2010). A Meta-Analysis of the Impact of Short-Term Sleep Deprivation on Cognitive Variables. *Psychological Bulletin, 136*(3), 375-389. doi: 10.1037/a0018883

Liu, Zhong-Wu, Faraguna, Ugo, Cirelli, Chiara, Tononi, Giulio, & Gao, Xiao-Bing. (2010). Direct Evidence for Wake-Related Increases and Sleep-Related Decreases in Synaptic Strength in Rodent Cortex. *The Journal of Neuroscience, 30*(25), 8671– 8675 doi: 10.1523/JNEUROSCI.1409-10.2010

Lucka, S. J., Woodmana, G. F., & Vogela, E. K. (2000). Event-related potential studies of attention. *Trends in Cognitive Sciences, 4*(11), 432-440. doi: 10.1016/S1364-6613(00)01545-X

Mardi, Z., Ashtiani, S. N., & Mikaili, M. (2011). EEG-based Drowsiness Detection for Safe Driving Using Chaotic Features and Statistical Tests. *Journal of medical signals and sensors, 1*(2), 130-137.

Maret, S., Faraguna, U., Nelson, A. B., Cirelli, C., & Tononi, G. (2011). Sleep and waking modulate spine turnover in the adolescent mouse cortex. *Nature Neuroscience, 14*(11), 1418-1420. doi: 10.1038/nn.2934

Mongrain, V., Hernandez, S. A., Pradervand, S., Dorsaz, S., Curie, T., Hagiwara, G., . . . Franken, P. (2010). Separating the contribution of glucocorticoids and wakefulness to the molecular and electrophysiological correlates of sleep homeostasis. *Sleep, 33*(9), 1147-1157.

Montgomery, S. A., & Asberg, M. (1979). A new depression scale designed to be sensitive to change. *The British journal of psychiatry: the journal of mental health, 134*, 382-389.

Ohe, Christina G. von der, Garner, Craig C., Darian-Smith, Corinna, & Heller, H. Craig. (2007). Synaptic Protein Dynamics in Hibernation. *The Journal of Neuroscience, 27*(1), 84-92. doi: 10.1523/JNEUROSCI.4385-06.2007

Olijslagers, J. E., Kloet, E. R. de, Elgersma, Y., Woerden, G. M. van, Joëls, M., & Karst, H. (2008). Rapid changes in hippocampal CA1 pyramidal cell function via pre- as well as postsynaptic membrane mineralocorticoid receptors. *The European journal of neuroscience, 27*(10), 2542-2550. doi: 10.1111/j.1460-9568.2008.06220.x.

Orzel-Gryglewska, Jolanta. (2010). Consequences of sleep deprivation *International Journal of Occupational Medicine and Environmental Health, 23*(1), 95-114. doi: 10.2478/v10001-010-0004-9

Peng, I-Feng, Berke, Brett A., Zhu, Yue, Lee, Wei-Hua, Chen, Wenjia, & Wu, Chun-Fang. (2007). Temperature-Dependent Developmental Plasticity of Drosophila Neurons: Cell-Autonomous Roles of Membrane Excitability, Ca2+ Influx, and cAMP Signaling. *The Journal of Neuroscience, 27*(46), 12611–12622. doi: 10.1523/JNEUROSCI.2179-07.2007

Rasch, B., Büchel, C., Gais, S., & Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. *Science, 315*(5817), 1426-1429.

Rechtschaffen, Allan, & Bergmann, Bernard M. (1995). Sleep deprivation in the rat by the disk-over-water method. *Behavioural Brain Research, 69*(1-2), 55-63.

Reid, Kathryn J., Martinovich, Zoran, Finkel, Sanford, Statsinger, Judy, Golden, Robyn, Harter, Kathryne, & Zee, Phyllis C. (2006). Sleep: A marker of physical and mental health in the elderly. *American Journal of Geriatric Psychiatry, 14*(10). doi: 10.1097/01.JGP.0000206164.56404.ba

Reinoso-Suarez, Fernando, Andres, Isabel de, Rodrigo-Angulo, Margarita L., & Garzon, Miguel. (2001). Brain structures and mechanisms involved in the generation of REM sleep. *Sleep medicine reviews, 5*(1), 63-77. doi: 10.1053/smrv.2000.0136

Reuter, Martin, Schmansky, Nicholas J., Rosas, H. Diana, & Fischl, Bruce. (2012). Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage, 61*(4). doi: 10.1016/j.neuroimage.2012.02.084

Ross, John J. (1965). Neurological Findings After Prolonged Sleep Deprivation. *Archives of neurology, 12*(4), 399-403. doi: 10.1001/archneur.1965.00460280069006

Sagi, Y., Tavor, I., Hofstetter, S., Tzur-Moryosef, S., Blumenfeld-Katzir, T., & Assaf, Y. (2012). Learning in the fast lane: new insights into neuroplasticity. *Neuron, 73*(6), 1195-1203. doi: 10.1016/j.neuron.2012.01.025

Sandyk, R., Tsagas, N., Anninos, P. A., & Derpapas, K. (1992). Magnetic fields mimic the behavioral effects of REM sleep deprivation in humans. *International journal of neuroscience, 65*(1-4), 61-68.

Siegel, Jerome M. (2005). Clues to the functions of mammalian sleep. *Nature, 437*(27), 1264-1271. doi: 10.1038/nature04285

Siegel, Jerome M. (2009). Sleep viewed as a state of adaptive inactivity. *Nature Reviews Neuroscience, 10*(10), 747-753. doi: 10.1038/nrn2697

Siegel, Jerome M. (2012). Suppression of Sleep for Mating. *Science, 337*(6102), 1610-1611. doi: 10.1126/science.1228466

Stevenson, R. D. (1985). Body Size and Limits to the Daily Range of Body Temperature in Terrestrial Ectotherms. *The American Naturalist, 125*(1), 102-117.

Stickgold, Robert. (2006). Neuroscience: A memory boost while you sleep. *Nature, 444*(7119), 559-560. doi: 10.1038/nature05309

Streitbürger, D. P., Möller, H. E., Tittgemeyer, M., Hund-Georgiadis, M., Schroeter, M. L., & Mueller, K. (2012). Investigating Structural Brain Changes of Dehydration Using Voxel-Based Morphometry. *PLOS ONE, 7*(8), e44195. doi: 10.1371/journal.pone.0044195

Taheri, S., Lin, L., Austin, D., Young, T., & Mignot, E. (2004). Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLOS Medicine, 1*(3), 210-217. doi: 10.1371/journal.pmed.0010062

Tononi, Giulio, & Cirelli, Chiara. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain research bulletin, 62*(2), 143-150.

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

Tononi, Giulio, & Cirelli, Chiara. (2012). Time to be SHY? Some comments on sleep and synaptic homeostasis. *Neural Plasticity, 2012*(415250), 1-12. doi: 10.1155/2012/415250

Vassalli, Anne, & Dijk, Derk-Jan. (2009). Sleep function: current questions and new approaches. *European journal of neuroscience, 29*(9), 1830-1841. doi: 10.1111/j.1460-9568.2009.06767.x

Vertes, Robert P., & Eastman, Kathleen E. (2000). The case against memory consolidation in REM sleep. *Behavioral and brain sciences, 23*(6), 793–1121. doi: 10.1017/S0140525X00004003

Vyazovskiy, Vladyslav V, Olcese, Umberto, Lazimy, Yaniv M, Faraguna, Ugo, Esser, Steve K, Williams, Justin C, . . . Tononi, Giulio. (2009). Cortical firing and sleep homeostasis. *Neuron, 63*(6), 865–878. doi: 10.1016/j.neuron.2009.08.024

Wijsenbeek-Wijler, Henriette. (1976). *Aristotle's Concept of Soul, Sleep and Dreams*. Amsterdam: A.M. Hakkert.

Young, R. C., Biggs, J. T., Ziegler, V. E., & Meyer, D. A. (1978). A rating scale for mania: reliability, validity and sensitivity. *The British journal of psychiatry: the journal of mental health, 133*, 429-435.