University of Groningen

Bachelor's Thesis Life Sciences

The Connection between Sleep, Cognition and Synaptic Plasticity

Author:
Robin Pijnacker (s3715868)

Lecturer:
prof. dr. Robbert Havekes

June 27, 2021

Summary

All organisms spend a substantial part of their lifetime sleeping. However, the function of this unconscious behaviour remains elusive. This review aims to elucidate the effect of sleep on cognitive behavior and synaptic plasticity in the hippocampus of rodents. First, evidence is provided that shows a detrimental effect of sleep deprivation on the ability to learn. Since synaptic plasticity is regarded as the neural basis for learning and memory, the connection between synaptic plasticity and cognition is explained. An increase in dendritic spine number and density is associated with improved performance in learning and memory tasks. However, the effect of sleep on synaptic plasticity is still unknown. Two main theories are discussed: the synaptic homeostasis hypothesis, which states that sleep weakens synaptic strength, and the opposing hypothesis, which claims that synaptic strength is increased by sleep. Contradicting findings are examined and an attempt is made to explain the differences. Prior experiences are found to be an important component of sleep research, since the introduction of novel elements increases synaptic strength. Furthermore, the age of animals is of significance as well, since young adult animals have a higher elimination rate, which should not be mistaken for a downscaling effect of sleep. Lastly, emphasis is placed on the dynamic nature of sleep, as it should not be thought of as a uniform process.

Contents

1 Introduction

Sleep is a universal phenomenon of life. It can be observed in all organisms, ranging from fruit flies to monkeys. Although the characteristics of sleep can vary per animal, the act itself has been conserved throughout evolution. This is remarkable, since sleeping does not allow for the execution of essential activities, such as foraging for food, forming social connections or reproducing. This is no different in humans, since we spend a substantial part of our life in this unconscious state as well.

We manipulate the timing and duration of our sleep to prevent it from interfering with societal commitments. However, we have not yet discovered a way to live without it. If we stop sleeping, it would have a detrimental effect on our health and well-being. Indeed, a lack of sleep is a risk factor for many impairments, both physically and mentally. Sleep deprivation in humans is associated with a multitude of diseases such as obesity, diabetes and heart diseases (Cappuccio & Miller, 2017). Furthermore, inadequate sleep can also result in poor mental health. For example, people that suffer from insomnia have a higher likelihood of developing depression, anxiety, and forgetfulness (Scott et al., 2017). This indicates that there are serious problems associated with sleep disturbances and a loss of sleep. Sleep research attempts to get a better understanding about the process and the underlying mechanisms, with the aim to improve the quality of sleep.

The field of sleep research uses model organisms to get more insight into the physiological and molecular mechanisms behind sleep. The effect of sleep deprivation is examined by keeping animals, such as rodents, awake for a period of time where they would normally be asleep. One of the brain regions that is studied extensively using this method is the hippocampus. This region is particularly responsive to sleep deprivation. The hippocampus plays an important role in the consolidation from short-term to long-term memory and in spatial memory, and impaired sleep has a negative effect on the ability to acquire, store and retrieve memories. Clearly sleep must have some impact on brain structure that influences cognitive function. What is the effect of sleep on synaptic plasticity and how does this affect learning and memory?

Synaptic plasticity provides the neural basis for learning and memory. The formation of a memory requires strengthening of neuronal connections in the hippocampus. However, the way in which sleep affects this process is still elusive. One line of research shows that down-regulation of synaptic strength during sleep deprivation impairs the formation of memories. However, an alternative line of research demonstrates that sleep deprivation actually increases synaptic strength, and argues that synaptic connections are downregulated and renormalized during sleep instead of during sleep deprivation. This review attempts to elucidate the effect of sleep and sleep deprivation by comparing different studies and considering the methodological variations between them. First, an overview will be provided of the impact of sleep deprivation on the cognitive behaviour of animals. Then, evidence of both theories regarding synaptic plasticity will be given and the methods will be discussed. Finally, the molecular mechanisms underlying synaptic plasticity will be considered. Ultimately, this review tries to reconcile recent contradictory findings and provide clarification on the function of sleep.

Figure 1: What is the connection between sleep, learning and memory, and synaptic plasticity? Sleep (indicated in blue), learning and memory (indicated in yellow) and synaptic plasticity (indicated by a stylized synapse in red) are heavily connected. This review will discuss recent findings and examine the effect of sleep on cognitive behaviour and synaptic plasticity in the hippocampus of rodents. (The logos used in making this illustration were adapted from https://thenounproject.com.)

2 Sleep Deprivation Impairs Cognitive Memory

As stated in Section 1, sleep is crucial for memory acquisition, consolidation, and retrieval. The brain during sleep presumably goes over the events that occurred throughout the day, and makes sure they are processed correctly. In humans, sleep deprivation is already known to cause forgetfulness. This is also the case in rodents. In research using rodents, sleep deprivation is known to particularly affect the formation of memories that require the hippocampus (Prince et al., 2014). This is reflected in the ability of a rat or mouse to store information. The hippocampus regulates spatial memory, a process that enables a person or animal to remember different locations as well as spatial relations between objects. Tasks which require spatial memory are performed poorly by rats that are sleep deprived. This section will discuss research that examined the effect of sleep deprivation on learning and memory in mice and rats. It will also investigate if this effect is influenced by the task that animals need to perform. Lastly, it will examine the importance of the amount and the timing of sleep deprivation.

Contextual fear conditioning is an associative learning task in which a rat or mouse learns to associate an environment with a fear-inducing stimulus. The task requires spatial memory and therefore is regulated by the hippocampus. Prolonged sleep deprivation in rats is known to decrease cognitive performance in this task. Rats that were sleep deprived for three days, or for 20 hours a day during a three day period show a lack of contextual fear, as conditioned freezing and defecation were minimal (Ruskin & LaHoste, 2008). In a similar study, an equivalent effect is found. Sleep deprivation of multiple days prior to memory acquisition also caused impaired contextual fear conditioning (McDermott et al., 2003). Sleep deprivation of two days also had a significant negative effect on cognitive performance in the Morris water maze task, where animals in a swimming arena use environmental cues to navigate to a submerged platform (Hajali et al., 2015). However, sleep deprived rats do not perform

significantly worse in amygdala-mediated tone-cued fear condition, which is not hippocampus dependent (Ruskin et al., 2004). Thus, sleep deprivation for multiple days impairs cognitive performance in tasks mediated by the hippocampus and does not always impair hippocampus-independent functions.

Nevertheless, multiple day sleep deprivation is not required to see behavioural effects. A relatively short forced wakefulness is already sufficient for attenuated cognition. In the object-recognition task, animals are reintroduced to a previously explored environment after the placement of novel objects. Animals will show less interest in familiar objects if a novel object is introduced. This task is dependent on the hippocampus as well since recognition of novel object requires spatial memory. Spatial memory is impaired when sleep deprivation occurs immediately after acquisition. Rats that are sleep deprived for six hours immediately after the first trial do not show an increased exploration towards novel objects (Palchykova et al., 2006). Thus, rats have impaired cognition when they are sleep deprived for a relatively short time even after hippocampal-dependent tasks.

It is not always clear whether this effect is due to impaired acquisition and memory encoding or due to defective memory consolidation. Sleep prior to learning may affect memory processes as well. This was examined in the study of Hagewoud et al. (2010), which shows that 12 hours of sleep deprivation significantly impaired spatial working memory in a novel arm recognition task. The novel arm recognition task is similar to the object recognition task. In this task, rats can explore two of three interconnected arms forming a Y. In the test phase, all three arms are accessible. Rats that have a good spatial working memory will spend more time exploring the arm that was previously not accessible. 12 hours of sleep deprivation prior to learning significantly impaired spatial working memory in a novel arm recognition task. This suggests that sleep deprivation has an effect on memory acquisition of hippocampus dependent spatial memory tasks, even when sleep deprivation was induced prior to learning.

The importance of timing in sleep research is emphasized by the study of Prince et al. (2014), who show that three hours of sleep deprivation significantly impaired memory when deprivation began 1 hour after training. This is supported by a study where rats showed impaired cognition when they were sleep deprived for 6 hours immediately after learning (Palchykova et al., 2006). This was not the case in rats that were sleep deprived 6 hours later.

The effects that were found in the studies mentioned above are not likely to be a result of stress. Plasma levels of the stress hormone CORT were not significantly elevated in sleep deprived rodents.

Although the methods of sleep related research vary, the findings are relatively similar. The hippocampus regulates spatial working memory tasks. Performance of these tasks is impaired by sleep deprivation ranging from a few hours to multiple days. Furthermore, cognitive performance on these tasks is reduced either by sleep deprivation prior to or following the task. However, the timing of sleep deprivation following a task should be taken into account. Lastly, there is no evidence that sleep deprivation is related to improved cognition. Thus, sleep deprivation has a negative effect on performance of hippocampus dependent tasks, and sleep is necessary for a proper performance. Sleep improves cognitive performance by making certain modifications in the brain. To get a deeper insight into how sleep affects neuronal connections, there should be a clear understanding about how synaptic plasticity is normally related to learning and memory. Therefore, the next section will be devoted to explain the connection between cognition and the brain.

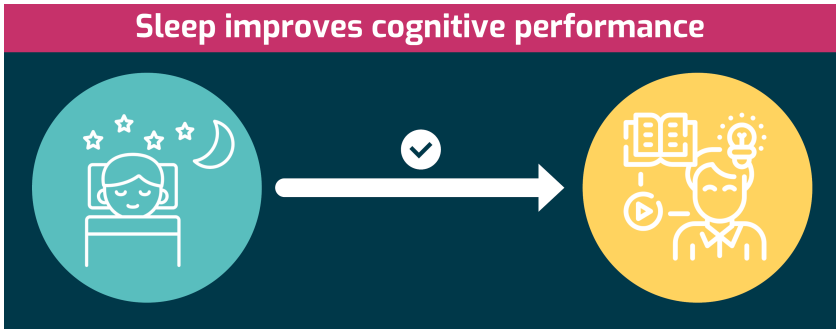

Figure 2: Sleep (indicated in blue) has a beneficial effect on learning and memory (indicated in yellow). Experimental research has shown that a lack of sleep results in poor cognitive performance. Especially the hippocampus, which regulates spatial memory, is affected negatively by sleep deprivation. Thus, sleep is essential for the improvement of cognition. (The logos used in making this illustration were adapted from https://thenounproject.com.)

3 Synaptic Plasticity and Cognition

What is known about cognition on a structural level? The formation of memory begins in the cortex. Signals are then transmitted from the sensory cortex to the hippocampus, which is involved in the formation of new memories. The hippocampus consists of neurons, which receive input from other cells at their dendrites. Dendritic spines are where some of the key processes that facilitate learning and memory take place (McCann & Ross, 2017). Dendritic spines create synapses with nearby axons. Synapses that are frequently used are strengthened, whereas synapses that are rarely used are eliminated. The growth of new synapses, pruning of old synapses and the change in existing synapses is called synaptic plasticity. Neurobiologists regard the proposition that synaptic plasticity provides the neural basis for learning and memory as almost self-evident (Bliss, 1979).

When memories are formed, neurons in the hippocampus are reactivated and are selectively strengthened. This allows you to remember and recall earlier experiences. A study by Van Reempts et al. (1992) showed that rats subjected to a one-way active avoidance task showed obvious shape changes in dendritic spines of the hippocampal supragranular molecular layer. This was paired with a significant improvement in performance in later training sessions. This shows that synaptic plasticity can be beneficial for learning and memory performance. If the connection between neurons is strengthened persistently this can ultimately lead to the formation of long-term memory. Evidence derived using optical imaging, molecular-genetic and optogenetic techniques continue to offer support for the idea that changing the strength of connections between neurons is one of the major mechanisms by which memories are stored in the brain (Takeuchi et al., 2014).

The acquisition of new memories is associated with the change in the density of dendritic spines, since an increase of dendritic spines is associated with improved cognition. Mice that learned motor tasks showed an increase in the formation of dendritic spines (Yang et al., 2014). Furthermore, there is a correlation between spine number and performance in the radial arm maze (Mahmmoud et al., 2015). In the study of Leuner et al. (2003) adult male rats were trained with an associative hippocampus-dependent learning task. Golgi staining allowed the assessment of spine density on the pyramidal cells of area CA1 of the hippocampus and revealed an increased density of dendritic spines after performing a hippocampus-dependent task. An increase in dendritic spines is paired with a higher potential for synaptic contact, suggesting that the density of dendrites is increased when there is a need for memory formation.

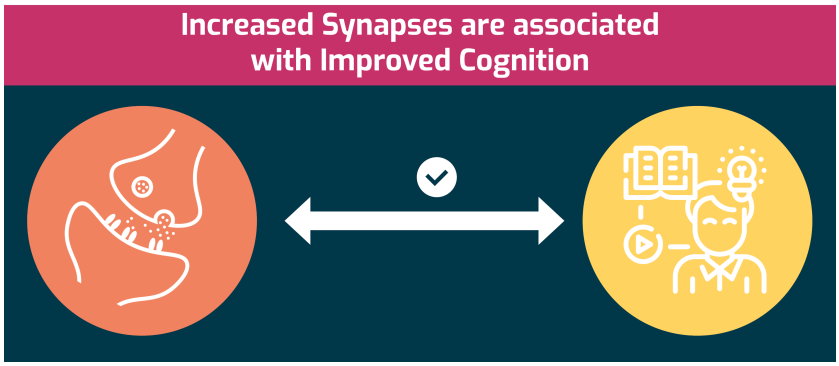

Figure 3: Increased spine density (indicated in orange) has a beneficial effect on learning and memory (indicated in yellow). Experimental research has shown that an increases in dendritic spines is associated with improved cognitive performance. On the other hand, performing learning and memory tasks increases synaptic strength. Therefore, there is a mutually beneficial relationship between synaptic plasticity and cognition. (The logos used in making this illustration were adapted from https://thenounproject.com.)

As discussed above, sleep is essential for memory consolidation since a lack of sleep can result in impaired spatial memory. It is widely acknowledged that sleep deprivation has a profound effect on synaptic consolidation as well, particularly for memories that require the hippocampus. It is unclear, however, what exactly happens on a structural level. Since sleep deprivation is associated with impaired cognition, and an increase in spine number and density is associated with improved cognition, it is a reasonable assumption that sleep deprivation impairs cognition by decreasing the number of spines in the hippocampus of rodents. Some researchers found that sleep deprivation indeed leads to reductions in spine density and synaptic strength. However, the synaptic homeostasis hypothesis claims the opposite and states that sleep deprivation leads to an increase in synaptic strength instead. The next section will explain these theories and provides supporting research for both hypotheses.

4 Sleep Deprivation and Synaptic Plasticity

4.1 Sleep Weakens Synaptic Strength

One of the main theories about what happens in the brain during sleep is the synaptic homeostasis hypothesis. This hypothesis claims that synaptic strength increases during wake and decreases during sleep (Tononi & Cirelli, 2016). A recent article by Cirelli & Tononi (2021) states that sleep must have an essential function that cannot take place during wakefulness. Otherwise, the need for sleep would have vanished throughout evolution. Cirelli & Tononi (2021) propose that sleep functions as the renormalization of synapses. This renormalization is needed as a compensation for the increase in synaptic connections during the day. The brain receives many inputs during wakefulness. New connections form throughout the day when acquiring new information and forming memories. These inputs strengthen and increase dendritic connections. However, the ongoing strengthening of synapses requires an overload of energy and would saturate the brain if no downscaling takes place, thus impairing the acquisition of new memories. Therefore, during sleep, the brain decreases the amount and strength of synaptic connections, maintaining synaptic homeostasis. As a result, according to the synaptic homeostasis hypothesis, synaptic strength should be biased towards net potentiation during waking and net downscaling during sleep.

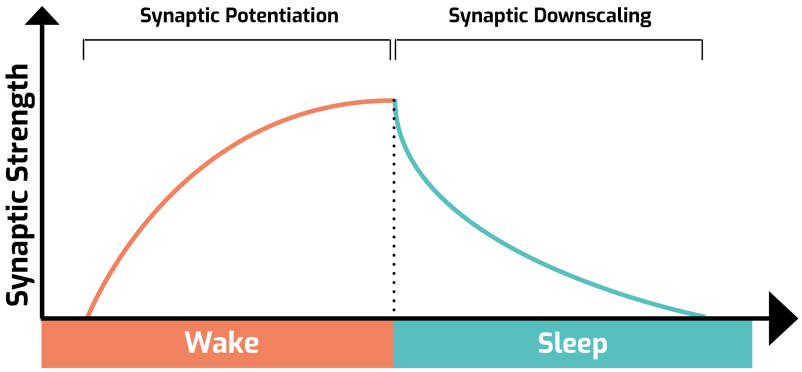

Figure 4: The synaptic homeostasis hypothesis states that synaptic strength should be biased towards net potentiation during wake and net downscaling during sleep. Inputs during wakefulness increase spine density. The decrease in synaptic connections during sleep serves as the renormalization of the number of synapses and therefore maintains synaptic homeostasis.

Experimental research provides evidence for the downscaling of synaptic strength during sleep. One measure of synaptic strength is the number of spines present in a particular brain region of an animal. In Drosophila neuronal circuits, synapse size or number increased after a few hours of wake and only decreased if flies were allowed to sleep. A richer wake experience results in both increased synaptic growth and a greater need for sleep, which shows the essential nature of the renormalization of spines (Bushey et al., 2011). In other research, two-photon microscopy is used, which creates an image of live cells. This method allows for the monitoring of the development of spines over time. Two-photon microscopy in adolescent mice revealed that waking results in a net increase in cortical spines, whereas sleep is associated with net spine loss (Maret et al., 2011). Not only the number of spines but also spine volume in the CA1 hippocampal region was significantly greater in sleep deprived mice (Gisabella et al., 2020). The underlying reason for the increase in connectivity is presumably a lower rate of elimination of dendritic spines during wakefulness compared with spine elimination during sleep (Yang & Gan, 2012).

Another morphological measure of synaptic strength is the axon-spine interface. This is the direct area of contact between pre-synapse and post-synapse and correlates with the area of postsynaptic density. According to the synaptic homeostasis hypothesis, the contact area between dendritic spines and axon terminals decreases during sleep and increases during wakefulness. Indeed, this was the case for a multitude of studies. In the primary motor and sensory cortex of mice, the axon spine interface was on average 18% lower after sleep compared with wakefulness (De Vivo et al., 2017). The axon spine interface of cortical synapses was also smaller after sleep in mouse pups, which shows that this effect is persistent through ages (de Vivo et al., 2019). A similar result was found when hippocampal spines were observed. The synapses in the hippocampus of mice that were sleep deprived showed an increased axon-spine interface together with an increased synapse density (Spano et al., 2019). Furthermore, a study by Cirelli & Tononi (2020) found interesting differences between the primary cortex and the CA1 region of the hippocampus. In two-week-old mouse pups, the decline in ASI size after sleep was larger, and affected more cortical synapses, compared with one-month-old adolescent mice. To summarize, research shows a net decrease in the number of spines and in the axon-spine interface in sleep compared to wakefulness, which confirms the synaptic homeostasis hypothesis.

4.2 Sleep Increases Synaptic Strength

Another line of research shows results that do not align with the synaptic homeostasis hypothesis. An alternative theory based on these studies states that sleep does not decrease synaptic strength but instead ensures the enhancement of it. This increase in synaptic strength is needed to facilitate memory consolidation after learning. Although evidence for the synaptic homeostasis hypothesis is compelling, an abundance of results shows the opposite. In these cases, a net potentiation during sleep and a net downscaling during wake or during sleep deprivation was found.

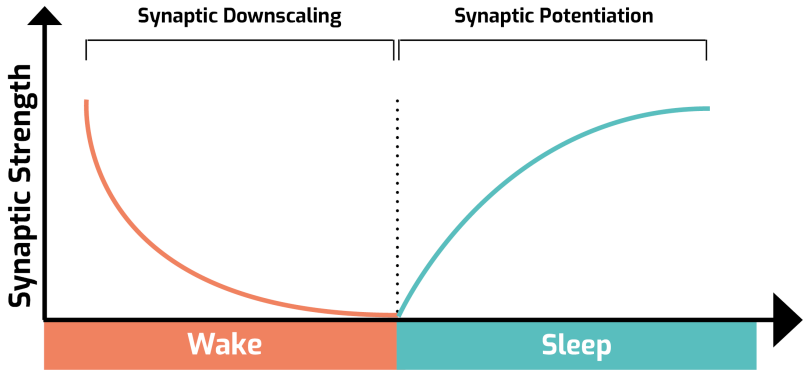

Figure 5: The alternative hypothesis states that synaptic strength should be biased towards net downscaling during wake and net potentiation during sleep. In contrast to the synaptic homeostasis hypothesis, this theory claims that the increase in synaptic strength during sleep is essential to maintain and strengthen connections needed for learning and memory.

Sleep deprivation can reduce dendritic spine number in the mammalian brain. Prolonged sleep deprivation of 24 hours does not affect dendritic length and branching length but reduces spine density in the CA1 of sleep deprived young adult rats (Acosta-peña et al., 2015). In this region, 30% of all subtypes of neurons also showed an attenuation with only five hours of sleep deprivation. In other cases, the attenuation of spine numbers was accompanied by a significant reduction in dendrite length (Havekes et al., 2016). A similar amount of sleep deprivation also reduces spine density in the dentate gyrus, especially for branched spines (Raven et al., 2019). If sleep deprivation is responsible for a decrease in spines, then this indicates that sleep would normally promote the formation of new spines. Indeed, sleep after learning facilitates the formation and protects the elimination of new dendritic spines on various sets of dendritic branches in response to learning tasks (Yang et al., 2014). REM sleep also strengthens and maintains newly formed spines, which are critical for behavioural improvement after learning (Li et al., 2017). Adult born neurons are also recruited by contextual fear learning and they are activated during REM sleep (Kumar et al., 2020). Furthermore, researchers have shown that sleep following a learning task enhanced connections in CA1 neurons of the hippocampus (Puentes-Mestril & Aton, 2017).

To summarize, sleep is not always associated with a reduction and the renormalization of spines. This is indicated by before mentioned studies, which show that not sleep, but rather sleep deprivation shows the attenuation of spine number and spine density. This is supported by research demonstrating that sleep promotes spine formation. Therefore, as visualized in Table 1 and Table 2, there is both evidence of synaptic potentiation as well as synaptic downscaling during sleep. How can these contributing findings be explained?

Species	Age	Brain Region	SD Method	Learning?	Result of sleep deprivation
Mice	23-44 Days	Barrel Cortex	Novel Objects	No	Increased synapse size or number (Maret et al., 2011)
Mice	3-4 Months	Hippocampus	Gentle Handling	No	Increased spine number (Gisabella et al., 2020)
Mice	3 Weeks	Barrel Cortex	Novel Objects	No	Increased spine density (Yang & Gan, 2012)
Mice	4 Weeks	M1 and S1	Novel Objects	No	Lowered rate of elimination of dendritic spines (De Vivo et al., 2017)
Mice	2 Weeks	M1	Novel Objects	No	Increased axon spine interface (de Vivo et al., 2019)
Mice	4 Weeks	Hippocampus	Novel Objects	No	Increased axon spine interface Increased synapse density (Spano et al., 2019)

Table 1: Examples of studies where sleep deprivation increases, and sleep decreases, synaptic strength.

Species	Age	Brain Region	SD Method	Learning?	Result of sleep / SD
Rats	3/22 Months	Hippocampus	Gentle Handling	No	SD reduced spine density (Acosta-peña et al., 2015)
Mice	8-12 Weeks	Hippocampus	Gentle Handling	No	SD reduced amount of neurons (Havekes et al., 2016)
Mice	3 Months	Hippocampus	Gentle Handling	No	SD reduced spine density (Raven et al., 2019)
Mice	4 Weeks	Motor Cortex	Gentle Handling	Yes	Sleep increased spine number (Yang et al., 2014)
Mice	4 Weeks	Motor Cortex	Gentle Handling	Yes	Sleep strengthens newly formed spines (Li et al., 2017)
Mice	8 Weeks	Hippocampus	X	Yes	Increased axon spine interface Sleep activates new neurons (Kumar et al., 2020)

Table 2: Examples of studies where sleep deprivation decreases, and sleep increases, synaptic strength.

5 Prior Experiences Influence Synaptic Plasticity

The data outlined above indicates that not all findings promote the synaptic homeostasis hypothesis. The contradicting results may be explained by differences in methods. Sleep deprivation is used in sleep research since the effect of the absence of sleep could reveal its function. There are various ways to do this. For example, the introduction of novel objects, tapping on the cage, touching the animal or placing the animal on a moving platform all ensure wakefulness. Research mentioned above can be divided into studies that used gentle handling and studies that used novel objects as a means to deprive animals of sleep. Gentle handling includes the gentle poking of animals with a soft brush or tapping the cage. This method prevents REM and non-REM sleep (Franken et al., 1991), without stressing the animals or including novel stimuli. The novel object method introduces novel stimuli to the animals, such as a running wheel, novel objects or unfamiliar food. This excites the animals, and their increased activity and exploring keeps them awake. Both methods are successful in sleep deprivation, and they will both result in impaired cognition when applied prior to or following a learning task. However, the effect of the sleep deprivation method on structural plasticity should be taken into consideration when structural plasticity is examined. The review of (Havekes & Aton, 2020) focuses on this. They address the issue that the novel object method was used in all cases where sleep deprivation induced an increase in synaptic strength. Research has shown that the placement of novel objects increases synaptic connections in the brains of rodents. Therefore, an increase in synaptic plasticity following novel object sleep deprivation is likely the consequence of the introduction of the objects and does not result from the lack of sleep itself. Furthermore, a reduced spine number and density after sleep deprivation was found in studies that used the gentle handling method. Gentle handling is not associated with the increase of connections in the brain, since the gentle handling method is developed to introduce as little stimuli as possible to keep the animal awake (Vecsey et al., 2013). Thus, when investigating the effect of sleep deprivation on synaptic plasticity, a sleep deprivation method should be used that does not introduce novel stimuli. Therefore novel objects should be avoided in this research.

Additionally, not only the method used for sleep deprivation but all events experienced by an animal influence synaptic plasticity. Sleep is not a process that uniformly decreases synapses, instead, it leads to the downscaling of a subset of synapses while strengthening others. The strengthening of synapses is thought to be beneficial for learning and memory. Synaptic plasticity can be affected differently depending on the experiences of the animal prior to sleep. For example, when an animal experiences a learning task, sleep will strengthen the synapses that are important for the consolidation of this novel memory. Therefore, selectively strengthening synapses during sleep is required for cognition. This is supported by research that showed the formation of new spines and the strengthening and activation of newly formed spines during sleep following a learning task. This is also supported by research that showed an increase in spine number and density following sleep deprivation using novel objects, since these changes were induced by novel stimuli, and therefore has a similar effect as a learning task. Furthermore, spine density is reduced after gentle handling, since this does not introduce new stimuli, and therefore strengthening of synapses is not needed. Indeed, five hours of sleep deprivation does not induce a change in the length or density of spines in the hippocampus when there was no need for memory consolidation (Brodin et al., 2021). Thus, sleep can both increase and decrease synaptic connections in the brain, depending on experiences prior to sleeping. While synaptic weakening may occur across sleep in the absence of learning, post-learning changes to network activity in the sleeping brain can support synaptic strengthening. However, this theory fails to explain why the study of Gisabella et al. (2020) found a greater density of spines in the hippocampus of sleep deprived mice when these mice were sleep

deprived using the gentle handling method and were not exposed to a learning task. This suggests that other factors affect synaptic plasticity as well.

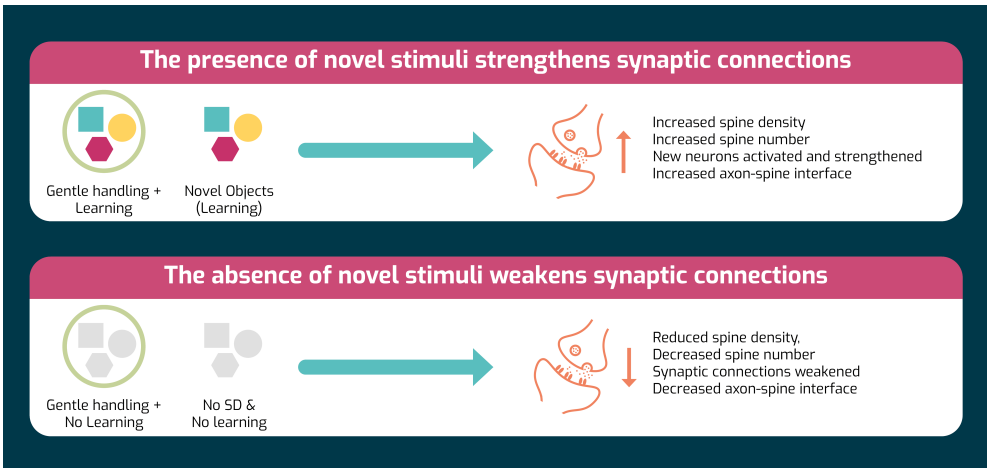

Figure 6: The presence of novel stimuli, such as a learning tasks or novel objects as a sleep deprivation method, strengthens synaptic connections. The formation of new spines and an increased axon-spine interface are necessary for the facilitation of memory consolidation after the introduction of novel inputs. In contrast, the absence of novel stimuli, such as gentle handling as a sleep deprivation method or the absence of a learning task, weakens synaptic connections. In these cases, there is no need for memory consolidation and therefore spine density and axon spine interfaced are decreased. (The logos used in making this illustration were adapted from https://thenounproject.com.)

6 Synapse Elimination Rate Differs Between Ages

One other methodological issue to take into consideration when investigating synaptic plasticity is the age of animals. As can be seen in Table 1 and Table 2, the research focused on examining the effect of sleep on cognitive behaviour and synaptic plasticity has used mainly adolescent animals of around four weeks old. In contrast, some of the other studies used adult animals (Gisabella et al., 2020; Acosta-peña et al., 2015; Havekes et al., 2016; Raven et al., 2019; Kumar et al., 2020). Therefore this review has made comparisons between studies that used animals of different ages, while synaptic plasticity differs between young adults and the elderly. Just like in humans, the brain of young adults is structured differently than that of adults, since age affects several properties of neurons and their connectivity. Age causes a progressive decline in cognitive function and synaptic plasticity (Konstantoudaki et al., 2018), specifically affecting the rate of synapse formation and elimination. In the cortex of young adolescent mice, 13%–20% of spines were eliminated in two weeks. This is a significantly higher removal than in adult mice, where in two weeks 3%–5% of spines were eliminated in various cortical regions (Zuo et al., 2005). Not only the rate of elimination varies between ages, the way different areas of the brain are affected by sleep deprivation changes as well. Spine density was reduced in the CA1 of sleep-deprived young adults, and interestingly, sleep deprivation increased spine density in PFC of aged animals. Furthermore, sleep affects animals of different ages in distinct ways. In young mice of three weeks, a few hours of sleep and wake can affect the density of cortical spines. However, this is not the case in adult mice, where spine turnover is limited and not impacted by sleep and wake (Maret et al., 2011)(Tononi & Cirelli, 2014).

Since the majority of studies supporting the synaptic homeostasis hypotheses used adolescent mice, this might give a biased idea about the overall impact of sleep deprivation. These findings support the idea that synaptic connections are weakened by sleep due to a higher elimination rate compared to wakefulness, whereas the elimination rate is already higher in young adult animals. The higher elimination rate that is found could therefore be a result of age, and not necessarily of sleep. In adult animals, the elimination rate is lower and sleep has a different impact on these animals. Therefore, the examination of these animals ultimately supports the idea that sleep does not decrease synaptic connections in the brain. To conclude, for an appropriate comparison between the studies described in this essay, animals of the same age should be used, since a higher elimination and therefore lower number of synapses could be attributed as the function of sleep while it is a characteristic of young adult age.

7 Discussion

Memory and synaptic plasticity are profoundly influenced by sleep. This essay aimed to provide an overview of what is currently understood about the interaction between these processes. It is widely acknowledged that sleep is beneficial for learning and memory since cognitive performance is reduced by sleep deprivation. Additionally, good performance in learning and memory tasks is associated with an increase in spine density and number. Consequently, a reasonable assumption would be that sleep increases cognitive performance by the upscaling of synaptic strength. Although the studies described in this essay showed that part of the available evidence supports this assumption, another line of research promotes the synaptic homeostasis hypothesis, which states that synaptic strengthening is weakened during sleep. Comparing gathered evidence reveals that the introduction of novel stimuli is a critical component whereof the effect needs to be considered when investigating the effect of sleep. While downscaling of synaptic strength is possible during sleep, the introduction of novel stimuli prioritizes the consolidation of memories, for which an increase in synapse number and density is needed. Thus, sleep is a dynamic process that can encourage the strengthening as well as the weakening of synaptic connections. It turns out that the assumption made earlier is not incorrect, since sleep indeed increases cognitive performance by the upscaling of synaptic strength. Which process occurs is dependent on the prior experiences of the animal. Moreover, the age of animals is of significance as well, since young adult animals have a higher spine elimination rate. Using young adult animals to examine the effect of sleep on synaptic strength therefore shows that sleep decreases spine number, while this is the consequence of age, and not sleep. Confusing the effect of methodological components as the effect of sleep is a recurring problem since the was also the case when using novel objects as a sleep deprivation method. Therefore, sleep research should make sure that the effect of method related components and the effect of sleep are separated from each other. Further research should also take into consideration that sleep is not a uniform process that always impacts the brain in the same way. Instead, the effect depends on what is necessary to process the events happening during the day.

Even when analyzing sleep in only one night, the effect is not consistent throughout the brain. Sleep can induce a change in plasticity that is distinct between regions of the brain. One characteristic of sleep, slow waves, are not distributed equally across the cortex. Instead, they can appear locally and asynchronously across brain regions (Siclari & Tononi, 2017). The reason that brain regions might be affected differently is because of their distinct functions. The hippocampus, for example, functions as a novelty detector, and CA1 neurons will therefore be more responsive to novelty than neurons in the primary cortex (Giovannini et al., 2001; VanElzakker et al., 2008). Moreover, the hippocampus itself is also not uniformly affected by sleep or a lack thereof. Where the amount of spines in the CA1 area of the

hippocampus is significantly reduced after five hours of sleep deprivation, this is not the case for spines in the CA3 area of the hippocampus. In that region, there does not seem to be any effect of sleep deprivation on dendrite length or spine number (Havekes et al., 2016). Even individual spines can react in distinct ways to sleep. To balance the number of spines, REM sleep eliminates some spines but also strengthens and maintains spines essential for improved learning and memory (Li et al., 2017). This illustrates that the effect of sleep can be highly diverse depending on the area of the brain, the region within that area and even on the level of individual spines, the effect will vary with experiences during wakefulness.

AMPA receptors make the effect of sleep even more distinct throughout the brain. These are ionotropic glutamate receptors that are present in a synapse. Following a stimulation, they bind with glutamate and are subsequently activated. AMPA receptors stay open for a prolonged time when the signal that stimulates the pre-synaptic neuron is stronger or persistent. This results in greater depolarization and indirectly causes NMDA receptors in the synapse to open. When signals are sustained, this can even create long term changes in the cell which results in long term potentiation. The amount of AMPA receptors are not equally distributed between synapses. Instead, additional AMPA receptors can be inserted into synapses, which is thought to contribute to synaptic potentiation during LTP. This is a critical process for the formation of hippocampus-dependent learning (Mitsushima et al., 2011). Sleep also plays a key role in the presence of AMPA receptors, since the level of receptors present in the hippocampus is decreased by sleep (Vyazovskiy et al., 2008). These molecular changes during post-learning sleep are associated with changes in cognitive performance after sleep (Miyamoto et al., 2021). However, AMPA receptors are abundant in mushroom spines, while few are included in thin spines and filopodia (Matsuzaki et al., 2001). Again, a variation on the level of spines makes it complicated to draw a universal conclusion about the effect of sleep on synaptic plasticity. Besides, there are additional molecular aspects of learning and memory that should be taken into consideration as well. To achieve an accurate view of sleep and implement this knowledge for the treatment of sleep loss related problems, future sleep research should recognize that sleep is an intricate and subtle process that does not have an overall function, but instead depends on numerous factors which should be examined in more detail.

References

Acosta-peña, E., Camacho-Abrego, I., Melgarejo-Gutiérrez, M., Flores, G., Drucker-Colín, R., & García-García, F. (2015). Sleep deprivation induces differential morphological changes in the hippocampus and prefrontal cortex in young and old rats. *Synapse*, 69(1), 15–25.

Bliss, T. (1979). Synaptic plasticity in the hippocampus. *Trends in Neurosciences*, 2, 42–45.

Brodin, A. T., Gabulya, S., Wellfelt, K., & Karlsson, T. E. (2021). Five hours total sleep deprivation does not affect ca1 dendritic length or spine density. *bioRxiv*.

Bushey, D., Tononi, G., & Cirelli, C. (2011). Sleep and synaptic homeostasis: structural evidence in drosophila. *Science*, 332(6037), 1576–1581.

Cappuccio, F. P. & Miller, M. A. (2017). Sleep and cardio-metabolic disease. *Current cardiology reports*, 19(11), 1–9.

Cirelli, C. & Tononi, G. (2020). Effects of sleep and waking on the synaptic ultrastructure. *Philosophical Transactions of the Royal Society B*, 375(1799), 20190235.

Cirelli, C. & Tononi, G. (2021). The why and how of sleep-dependent synaptic down-selection. In *Seminars in Cell & Developmental Biology*: Elsevier.

De Vivo, L., Bellesi, M., Marshall, W., Bushong, E. A., Ellisman, M. H., Tononi, G., & Cirelli, C. (2017). Ultrastructural evidence for synaptic scaling across the wake/sleep cycle. *Science*, 355(6324), 507–510.

de Vivo, L., Nagai, H., De Wispelaere, N., Spano, G. M., Marshall, W., Bellesi, M., Nemec, K. M., Schiereck, S. S., Nagai, M., Tononi, G., et al. (2019). Evidence for sleep-dependent synaptic renormalization in mouse pups. *Sleep*, 42(11), zsz184.

Franken, P., Dijk, D., Tobler, I., & Borbely, A. A. (1991). Sleep deprivation in rats: effects on eeg power spectra, vigilance states, and cortical temperature. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 261(1), R198–R208.

Giovannini, M., Rakovska, A., Benton, R., Pazzagli, M., Bianchi, L., & Pepeu, G. (2001). Effects of novelty and habituation on acetylcholine, gaba, and glutamate release from the frontal cortex and hippocampus of freely moving rats. *Neuroscience*, 106(1), 43–53.

Gisabella, B., Scammell, T., Bandaru, S. S., & Saper, C. B. (2020). Regulation of hippocampal dendritic spines following sleep deprivation. *Journal of Comparative Neurology*, 528(3), 380–388.

Hagewoud, R., Havekes, R., Novati, A., Keijser, J. N., Van der Zee, E. A., & Meerlo, P. (2010). Sleep deprivation impairs spatial working memory and reduces hippocampal ampa receptor phosphorylation. *Journal of sleep research*, 19(2), 280–288.

Hajali, V., Sheibani, V., Mahani, S. E., Hajializadeh, Z., Shabani, M., Aliabadi, H. P., Saadati, H., & Esmaeilpour, K. (2015). Ovariectomy does not exacerbate the negative effects of sleep deprivation on synaptic plasticity in rats. *Physiology & behavior*, 144, 73–81.

Havekes, R. & Aton, S. J. (2020). Impacts of sleep loss versus waking experience on brain plasticity: Parallel or orthogonal? *Trends in neurosciences*, 43(6), 385–393.

Havekes, R., Park, A. J., Tudor, J. C., Luczak, V. G., Hansen, R. T., Ferri, S. L., Bruinenberg, V. M., Poplawski, S. G., Day, J. P., Aton, S. J., et al. (2016). Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area ca1. *Elife*, 5, e13424.

Konstantoudaki, X., Chalkiadaki, K., Vasileiou, E., Kalemaki, K., Karagogeos, D., & Sidiropoulou, K. (2018). Prefrontal cortical-specific differences in behavior and synaptic plasticity between adolescent and adult mice. *Journal of neurophysiology*, 119(3), 822–833.

Kumar, D., Koyanagi, I., Carrier-Ruiz, A., Vergara, P., Srinivasan, S., Sugaya, Y., Kasuya, M., Yu, T.-S., Vogt, K. E., Muratani, M., et al. (2020). Sparse activity of hippocampal adult-born neurons during rem sleep is necessary for memory consolidation. *Neuron*, 107(3), 552–565.

Leuner, B., Falduto, J., & Shors, T. J. (2003). Associative memory formation increases the observation of dendritic spines in the hippocampus. *Journal of Neuroscience*, 23(2), 659–665.

Li, W., Ma, L., Yang, G., & Gan, W.-B. (2017). Rem sleep selectively prunes and maintains new synapses in development and learning. *Nature neuroscience*, 20(3), 427–437.

Mahmmoud, R. R., Sase, S., Aher, Y. D., Sase, A., Gröger, M., Mokhtar, M., Höger, H., & Lubec, G. (2015). Spatial and working memory is linked to spine density and mushroom spines. *PloS one*, 10(10), e0139739.

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.

Matsuzaki, M., Ellis-Davies, G. C., Nemoto, T., Miyashita, Y., Iino, M., & Kasai, H. (2001). Dendritic spine geometry is critical for ampa receptor expression in hippocampal ca1 pyramidal neurons. *Nature neuroscience*, 4(11), 1086–1092.

McCann, R. F. & Ross, D. A. (2017). A fragile balance: dendritic spines, learning, and memory. *Biological psychiatry*, 82(2), e11–e13.

McDermott, C. M., LaHoste, G. J., Chen, C., Musto, A., Bazan, N. G., & Magee, J. C. (2003). Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. *Journal of Neuroscience*, 23(29), 9687–9695.

Mitsushima, D., Ishihara, K., Sano, A., Kessels, H. W., & Takahashi, T. (2011). Contextual learning requires synaptic ampa receptor delivery in the hippocampus. *Proceedings of the National Academy of Sciences*, 108(30), 12503–12508.

Miyamoto, D., Marshall, W., Tononi, G., & Cirelli, C. (2021). Net decrease in spine-surface glua1-containing ampa receptors after post-learning sleep in the adult mouse cortex. *Nature communications*, 12(1), 1–13.

Palchykova, S., Winsky-Sommerer, R., Meerlo, P., Dürr, R., & Tobler, I. (2006). Sleep deprivation impairs object recognition in mice. *Neurobiology of learning and memory*, 85(3), 263–271.

Prince, T.-M., Wimmer, M., Choi, J., Havekes, R., Aton, S., & Abel, T. (2014). Sleep deprivation during a specific 3-hour time window post-training impairs hippocampal synaptic plasticity and memory. *Neurobiology of learning and memory*, 109, 122–130.

Puentes-Mestril, C. & Aton, S. J. (2017). Linking network activity to synaptic plasticity during sleep: hypotheses and recent data. *Frontiers in neural circuits*, 11, 61.

Raven, F., Meerlo, P., Van der Zee, E. A., Abel, T., & Havekes, R. (2019). A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice. *Neurobiology of learning and memory*, 160, 83–90.

Ruskin, D. N. & LaHoste, G. J. (2008). Aspects of learned fear related to the hippocampus are sleep-dependent. *Behavioural brain research*, 191(1), 67–71.

Ruskin, D. N., Liu, C., Dunn, K. E., Bazan, N. G., & LaHoste, G. J. (2004). Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. *European Journal of Neuroscience*, 19(11), 3121–3124.

Scott, A. J., Webb, T. L., & Rowse, G. (2017). Does improving sleep lead to better mental health? a protocol for a meta-analytic review of randomised controlled trials. *BMJ open*, 7(9).

Siclari, F. & Tononi, G. (2017). Local aspects of sleep and wakefulness. *Current opinion in neurobiology*, 44, 222–227.

Spano, G. M., Banningh, S. W., Marshall, W., de Vivo, L., Bellesi, M., Loschky, S. S., Tononi, G., & Cirelli, C. (2019). Sleep deprivation by exposure to novel objects increases synapse density and axon–spine interface in the hippocampal ca1 region of adolescent mice. *Journal of Neuroscience*, 39(34), 6613–6625.

Takeuchi, T., Duszkiewicz, A. J., & Morris, R. G. (2014). The synaptic plasticity and memory hypothesis: encoding, storage and persistence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 369(1633), 20130288.

Tononi, G. & Cirelli, C. (2014). Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron*, 81(1), 12–34.

Tononi, G. & Cirelli, C. (2016). Sleep and synaptic down-selection. *Micro-, meso-and macro-dynamics of the brain*, (pp. 99–106).

Van Reempts, J., Dikova, M., Werbrouck, L., Clincke, G., & Borgers, M. (1992). Synaptic plasticity in rat hippocampus associated with learning. *Behavioural brain research*, 51(2), 179–183.

VanElzakker, M., Fevurly, R. D., Breindel, T., & Spencer, R. L. (2008). Environmental novelty is associated with a selective increase in fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learning and Memory*, 15(12), 899—-908.

Vecsey, C. G., Wimmer, M. E., Havekes, R., Park, A. J., Perron, I. J., Meerlo, P., & Abel, T. (2013). Daily acclimation handling does not affect hippocampal long-term potentiation or cause chronic sleep deprivation in mice. *Sleep*, 36(4), 601–607.

Vyazovskiy, V. V., Cirelli, C., Pfister-Genskow, M., Faraguna, U., & Tononi, G. (2008). Molecular and electrophysiological evidence for net synaptic potentiation in wake and depression in sleep. *Nature neuroscience*, 11(2), 200–208.

Yang, G. & Gan, W.-B. (2012). Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. *Developmental neurobiology*, 72(11), 1391–1398.

Yang, G., Lai, C. S. W., Cichon, J., Ma, L., Li, W., & Gan, W.-B. (2014). Sleep promotes branch-specific formation of dendritic spines after learning. *Science*, 344(6188), 1173–1178.

Zuo, Y., Lin, A., Chang, P., & Gan, W.-B. (2005). Development of long-term dendritic spine stability in diverse regions of cerebral cortex. *Neuron*, 46(2), 181–189.