The effect of sleep deprivation on pain

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Image:https://www.freepik.com/premium-vector/sleepless-woman-lying-bed-with-fatigue_9379681.htm

Summary

Patients with diseases such as fibromyalgia (FM), rheumatoid arthritis (RA) and lower back pain struggle with a constant pain feeling that does not fade, day and night. In chronic pain disorders sleep complaints are very common. Subsequently, poor sleep can result in sleep deprivation. Numerous studies have investigated the effect of sleep deprivation on pain. These studies have indicated that sleep deprivation has a lowering effect on the pain threshold. To build towards the conclusion of what effect sleep deprivation has on pain, studies conduct experiments with either humans (clinical) or animals, especially rats. Subjects were sleep deprived and subsequently their pain threshold was measured. Overall the studies and the pathways conclude that after a night of sleep deprivation, participants were more sensitive to pain than fully rested patients. Pathways mentioned in this review overall supported the conclusion of more sensitivity to pain after being sleep deprived, although some contradictions were found. It is important to further conduct research about both the complex processes of pain and sleep, focusing on one aspect at a time.

Table of contents

Abbreviations

EEG	Electroencephalography
FM	Fibromyalgia
PWL	Paw Withdrawal Latency
NAc	Nucleus Accumbens
NREM	Non-Rapid Eye Movements
RA	Rheumatoid Arthritis
REM	Rapid Eye Movements
SWS	Slow Wave Sleep
QST	Quantitative Sensory Testing

1. Introduction

Everybody knows the feeling of accidently cutting themselves while cooking. A sharp pain enters the body and you flinch. After the cut is closed off with a band-aid it is quickly forgotten as the pain fades away. Unfortunately, this is not the case for patients who are constantly in a painful experience. Patients with diseases such as fibromyalgia (FM), rheumatoid arthritis (RA) and lower back pain [1,2] struggle with a constant pain feeling that does not fade, day and night. FM patients experience difficulties falling or staying asleep and waking up early in the morning. Furthermore, 90% of FM patients report disturbed and nonrestorative sleep [3].

Pain is defined by the International Association for the Study of Pain as "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [4]. Pain is a complex subject, considering it is subjective to every individual. Human beings discover what their definition of pain is throughout life [4]. They are able to communicate their pain, while this is not the case for animals, non-communicating humans and babies. Pain can be divided into chronic and acute. Chronic pain lingers longer, while acute pain is the one that fades more quickly. However, when acute pain is perceived for a longer period of time it can transform into chronic pain [5]. It is unclear why chronic pain is more developed in some patients when compared to others. Factors such as extent of injury, occupation and education levels do not contribute to a higher level of pain perception [6]. Since it has been established that previous mentioned factors do not contribute to a higher level of pain perception, the question raises what factors do.

To get a better understanding of what factors influence the pain perception, a closer look will be taken into the processes behind pain. As a person accidentally cuts themselves with a kitchen knife, nerve cells will sense that a damaging stimulus is brought to the body. The result of this stimulus is the feeling perceived as pain. It is the mechanism to prevent the event from happening again [7]. Pain sensation occurs through several mechanisms, the nociceptive mechanism being the most important [8]. Nociception is the process that deals with protective reflexes. The brain processes these noxious events, including tissue damage and injury [7]. When a cell is damaged as the result of injury, it can release substances that open up ion channels on nociceptor membranes [9]. The resulting feeling of pain has a negative influence on many processes in the human body, such as emotions, energy and it causes fatigue [1]. One of the more drastic effects is that RA and FM patients experience poor sleep due to a constant painful experience. In chronic pain disorders sleep complaints are very common, occurring from 67% to 88% of patients [10,11]. Subsequently, poor sleep can result in sleep deprivation [12]. Studies have shown that pain increases the sleep deprivation, which consequently lowers the pain threshold [12,13]. Hence, a vicious circle is created, where pain and sleep worsen each other, as pictured in figure 1.

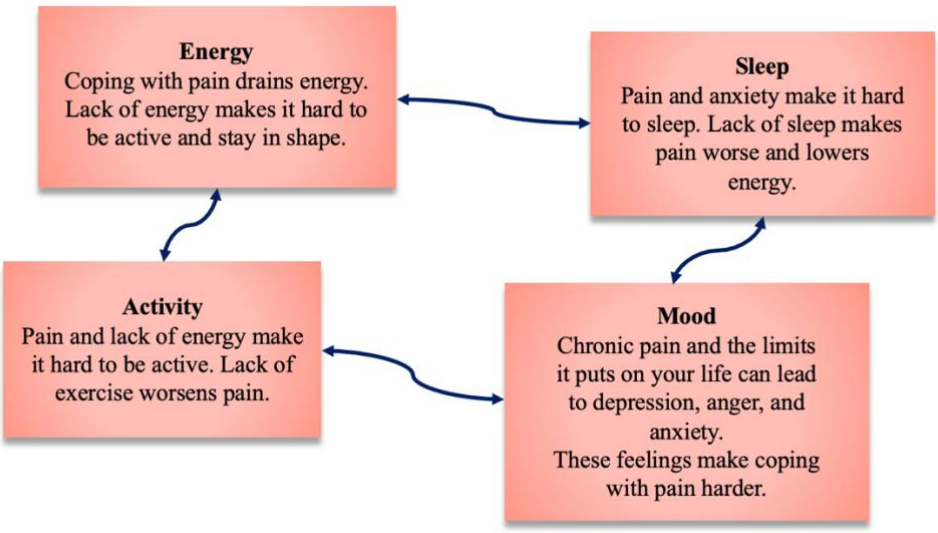

Figure 1: The bidirectional relationship of pain and sleep as adapted by St. Luke's[14]

The purpose of this thesis is to review literature wherein experiments have been conducted about the effect of sleep deprivation on the perception of pain. First, a short and deeper explanation will be given about sleep and its relationship to pain. Next, literature will be analyzed to answer the question what effect sleep deprivation has on pain.

Sleep is an important process, reducing the risk of a wide range of disorders, such as hypertension, obesity, cardiovascular disease, and neurodegeneration [15]. The different stages of sleep consist of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM consists of the transfer from wakefulness to light sleep, to finally a deep sleep, also called Slow Wave Sleep (SWS) [16,17]. A disrupted sleep pattern is characterized by a higher alpha Electroencephalography (EEG) activity, while normally NREM sleep is characterized by slow EEG rhythms [18,19]. Two studies established that sleep is disrupted with rheumatoid arthritis (RA) and fibromyalgia patients [18,20]. In the study of Drewes et al [20] RA patients participated, together with healthy subjects for comparison. There were no complaints of pain, sleepiness or tiredness among the healthy participants. Patients with RA however had trouble to fall asleep and therefore sleep problems occurred. Furthermore, there was no regenerative sleep pattern and during the day the patients experienced sleepiness. The conclusion that the authors drew was that patients with RA have a harder time falling asleep than healthy people with no sleep disturbances. This is likely due to the constant pain sensation. The disruption of sleep can also be felt by patients with fibromyalgia, as confirmed by several other studies [21-24]. A clinical human study, performed by Steinmiller et al, supports the hypothesis that pain has a negative effect on sleep by showing that a reduction of codeine (a component of opium poppy and a pain relieving drug [25]) resulted in an increase in daytime sleepiness [26].

Since it is still unknown what mechanisms are responsible for the effects that sleep deprivation and pain have on each other, the most important pathways where sleep deprivation and pain are connected will be highlighted in this thesis. Finally, possible gaps

and limitations in the studies will be described and a recommendation will be given for future research.

First it is important to review clinical and animal studies to gain information about the effect that sleep deprivation has on pain, which will be done in the next chapter.

2. Clinical and animal studies

Numerous studies have investigated the effect of sleep deprivation on pain. These studies have indicated that sleep deprivation has a lowering effect on the pain threshold. Studies conduct experiments with either humans (clinical) or animals, especially rats. Both clinical and animal experiments will be analyzed to determine what effects sleep deprivation has on the pain threshold.

2.1 Clinical studies

Clinical experiments are important to obtain more information about what effect sleep deprivation has on pain. Three studies have indicated that sleep deprivation has a lowering effect on the pain threshold. A study conducted by Krause et al [27], hypothesized that acute sleep deprivation will lower the thermal pain threshold and thus increase the range of pain sensation. The in-laboratory investigation consisted of healthy adult participants who were either given a full night of sleep for approximately 8 hours, or were deprived of sleep for one full night. Sleep deprivation was achieved by continuously waking the participants, playing games and walking around. To assess the pain perception, a thermal pain sensitivity assessment was conducted, in which the minimum painful temperature was measured, shown in figure 2. The results of the study showed that sleep deprived subjects experienced temperature pain at a significantly lower temperature than sleep rested subjects.

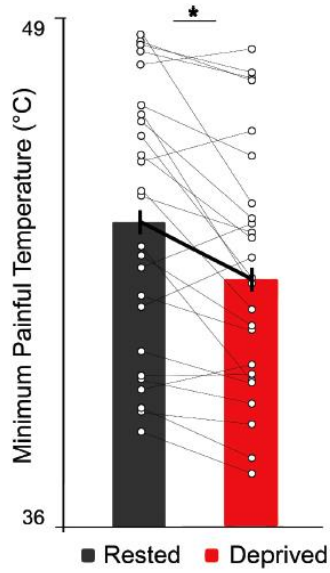

Figure 2: The minimum painful temperature in degrees Celsius plotted to rested and sleep deprived participants. When rested, participants experienced a higher painful temperature feeling than sleep deprived participants. (Sleep rested mean = 43.89°C [3.49°C s.d.], Sleep deprived mean = 42.47°C 903 [3.22°C s.d.] *P<0.0002) [27].

The clinical research of Hakki Onen et al confirms the findings of the study conducted by Krause and colleagues [28]. However, Hakki Onen and his team used a different type of method to assess pain in subjects. The method consisted of monitoring the subjects' sleep using polysomnography and testing the mechanical pain tolerance in healthy adults. The subjects in the clinical study of Hakki Onen et al were either deprived of SWS or REM sleep for the entirety of the night, for 6 consecutive nights. These 6 nights consisted of: N1 Adaptation

(undisturbed sleep, electrodes in place but recording not performed); N2 Baseline (undisturbed sleep, polysomnography); N3 Total sleep deprivation (with two observers); N4 SWS or REM sleep interruption-1 (on-line polysomnography); N5 SWS or REM sleep interruption-2 (on-line polysomnography); N6 Recovery (undisturbed sleep, polysomnography). SWS stage 3 & 4 were characterized by delta EEG waves. Auditory stimuli or physical stimulation were used to wake the subjects from their SWS sleep when one-third of the screen contained delta waves. REM sleep was characterized by low amplitude submental EMG, desynchronized EEG and the absence of both K-complexes and sleep spindles. When one-third of the screen contained the REM sleep characteristics, the subject would be awakened the same way as with SWS sleep. Hakki Onen et al state that as SWS increased, the average normalized pain tolerance score raised, shown in Figure 3. Furthermore, the group found that total sleep deprivation leads to a lower mechanical pain tolerance than selective sleep stage deprivation.

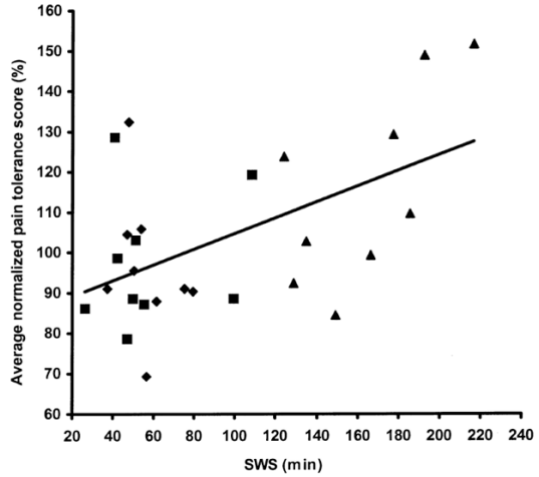

Figure 3: SWS is positively correlated with the average normalized pain tolerance [28].

Finally, the clinical research of Schuh-Hofer et al [29] compared subjects who were either given habitual sleep (no interruption of sleep) or were deprived of sleep for one whole night. Sleep deprivation was achieved by continuously waking the participants. Quantitative Sensory Testing (QST) was used as the method to assess the nociceptive thresholds. As shown in figure 4, sleep deprivation affects the majority of the nociceptive parameters: subjects who were sleep deprived were more sensitive to the QST tests than those who were given habitual sleep. In other words, the participants were more sensitive to pain after being sleep deprived.

Altogether, the clinical studies conclude that patients who experienced a night of sleep deprivation were more sensitive to pain than fully rested patients. To further strengthen this possible conclusion, animal studies were reviewed in the next paragraph.

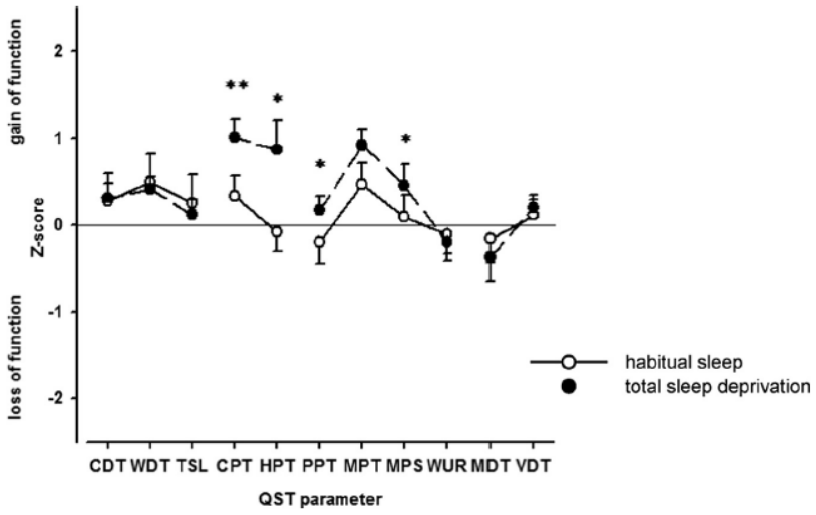

Figure 4: QST comparing habitual sleep and total sleep deprivation. A Z-score means an increase in sensitivity to that specific test. Error bars indicate standard deviation of the mean (SEM).**P < 0.01; *P < 0.05 (2-tailed paired Student t test) CDT (cold detection threshold), WDT (warm detection threshold), TSL (thermal sensory limen), CPT (cold pressure threshold), HPT (heat pain threshold), MDT (mechanical detection threshold), MPT (mechanical pain threshold), MPS (mechanical pain sensitivity) including procedures to evaluate dynamic mechanical allodynia (DMA), WUR (wind-up ratio), VDT (vibration threshold) and PPT (pressure pain threshold) [29].

2.2 Animal studies

Clinical studies can be limited in methods because of ethics, making it impossible to incorporate mechanical aspects into the experiments. Furthermore, the possible side effects of administered drugs can be harmful to humans. Studies assess pain in all species by researching the nociceptive behavior [7]. As mentioned earlier, while humans are able to communicate about their pain verbally pain can only be assessed in animals by their nociceptive behavior.

Besides an experiment on humans, Hakki Onen and colleagues performed a research on animals [30]. The research group described how rats were deprived of sleep using the 'inverted flower pot' technique, in which a rat is placed on a small platform ('flower pot') inside of a bucket with water. The technique prevents REM sleep by utilizing the muscular atonia that occurs when a rat subject transfers into REM sleep. It will result in the rat falling from the platform and subsequently waking up. For the control group, larger platforms were used to allow total relaxation. Mechanical, thermal, electrical, and chemical noxious stimuli were applied on the rats. Included were the paw pressure test, warm water tail-immersion test, tail electric shock test and the formalin test. These experiments were conducted over a 5-day period. The first two days were used to secure the baseline in a dry environment. The third day was used as an adaption period for the animals to get used to the transition of dry to wet environment. Finally, REM sleep deprivation was elicited on the last two days. The results of the research state that the animals showed a significant increase in the behavioral manifestations of pain to the mechanical, thermal and electrical noxious stimuli. However, no difference was found in the noxious chemical stimulus (formalin) after REM sleep deprivation.

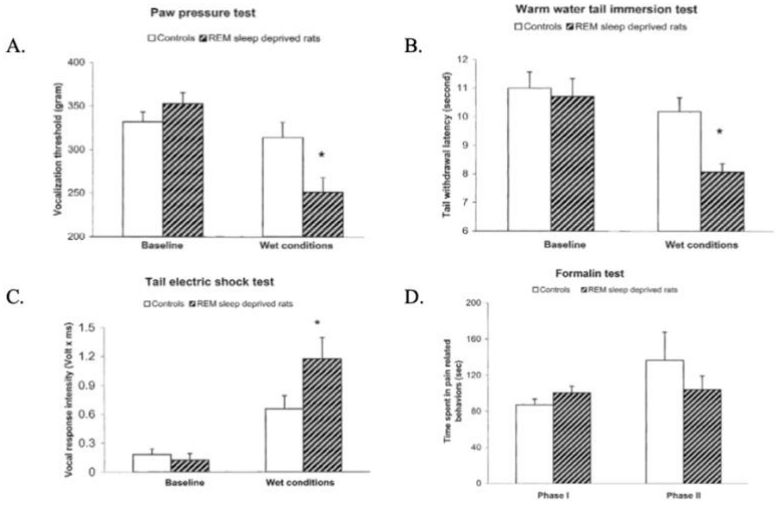

Figure 5: The assessment of nociception with different methods, comparing control to sleep deprivation. n = 8 for each animal group. A. The effect of sleep deprivation on mechanical pain threshold using the paw pressure test. Each bar represents the average score of the pain assessment test for two days. A decreased pain threshold was observed in the sleep deprived animals *P=0.005 (ANOVA, PLSD-Fisher test). B. The thermal pain threshold assessed using the warm water tail immersion test. The columns represent the average value of the pain score for a period of 2 days. Baseline shows no significant difference between control and SD. Under wet conditions there is a significant decrease in pain threshold *P=0.006 (ANOVA, PLSD-Fisher test). C. Vocal responses assessment with the tail electric shock test. Every bar stands for the mean of the 2nd peep envelopes. No significant differences were observed in baseline measurements, while under wet conditions the REM sleep deprivation resulted in a significant increase in intensity of the vocal responses. *P=0.009 (ANOVA, PLSD-Fisher test). D. Pain-related behavior was measured using the formalin test. No statistical difference was established between the sleep deprived and control group in both phase 1 (0–5 min period after formalin injection) and phase 2 (20–30 min period after formalin injection) (ANOVA, PLSD-Fisher test) [30].

A related study, conducted by Vanini et al, confirmed the findings of the research group of Hakki Onen [31]. The author hypothesized that acute sleep loss prior to noxious inflammatory insult will worsen the post-insult level of pain. Rats were either deprived of sleep completely or left to fully rest for a period of 21 days, depicted in figure 6. Rats received a subcutaneous microinjection of formalin to trigger pain. To achieve the sleep deprivation, the animals were kept awake by tapping on the side of the cage or stimulating the whiskers or tail whenever a sleep posture was observed.

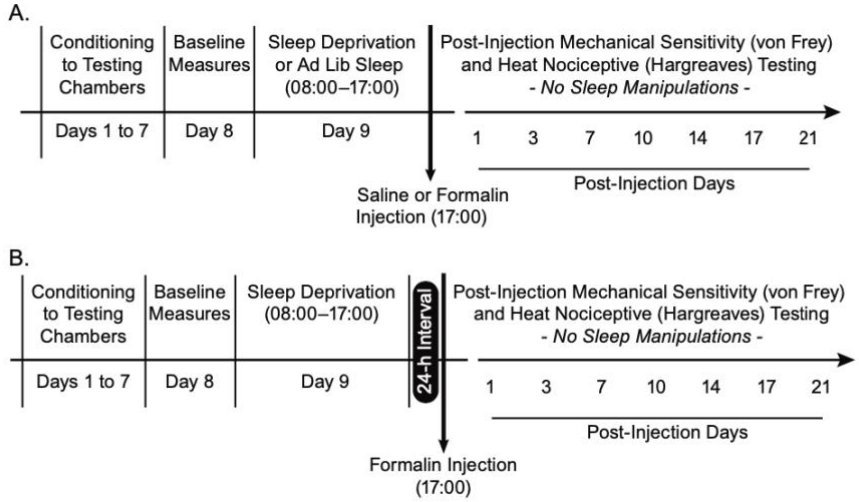

Figure 6 Experimental design of Vanini et al for quantifying the effect of sleep deprivation. A. Group where recovery sleep is included. B. Group wherein the formalin pain model is used to assess post-injection nociceptive levels [31].

The sleep deprived and sleep rested rat groups both received injections of either saline or formalin. The von Frey test was used to assess the mechanical sensitivity, which consists of an ascending stimulus in which an estimate can be made of the mechanical withdrawal threshold. To measure the thermal hyperalgesia the Hargreaves' paw withdrawal latency (PWL) method was used. Rodents were placed in a small space and a heat source would increase the heat through the glass floor until the animal started showing nociceptive behavior. As seen in figure 7 I, relative to *ad libitum* sleep + formalin group, the sleep deprivation + formalin resulted in a significant decrease in the mechanical threshold in the injected paw on day 3, 7 and 21 post-injection. Secondly, as seen in figure 7 II, compared to the *ad libitum* sleep + formalin group, sleep deprivation caused a significant lowering of the mechanical threshold in the non-injected paw on days 1, 3, 7, 10, and 21 post-injection. Furthermore, comparing the *ad libitum* sleep + saline to the *ad libitium* sleep + formalin, it increased the percentage of maximum possible effect (MPE) during day 1 and 3 post-injection. There was no longing effect of the thermal nociception due to an injection or the deprivation of sleep. Overall, the combination of sleep deprivation and formalin injection elicited an increase in nociceptive behavior in the subjects [32].

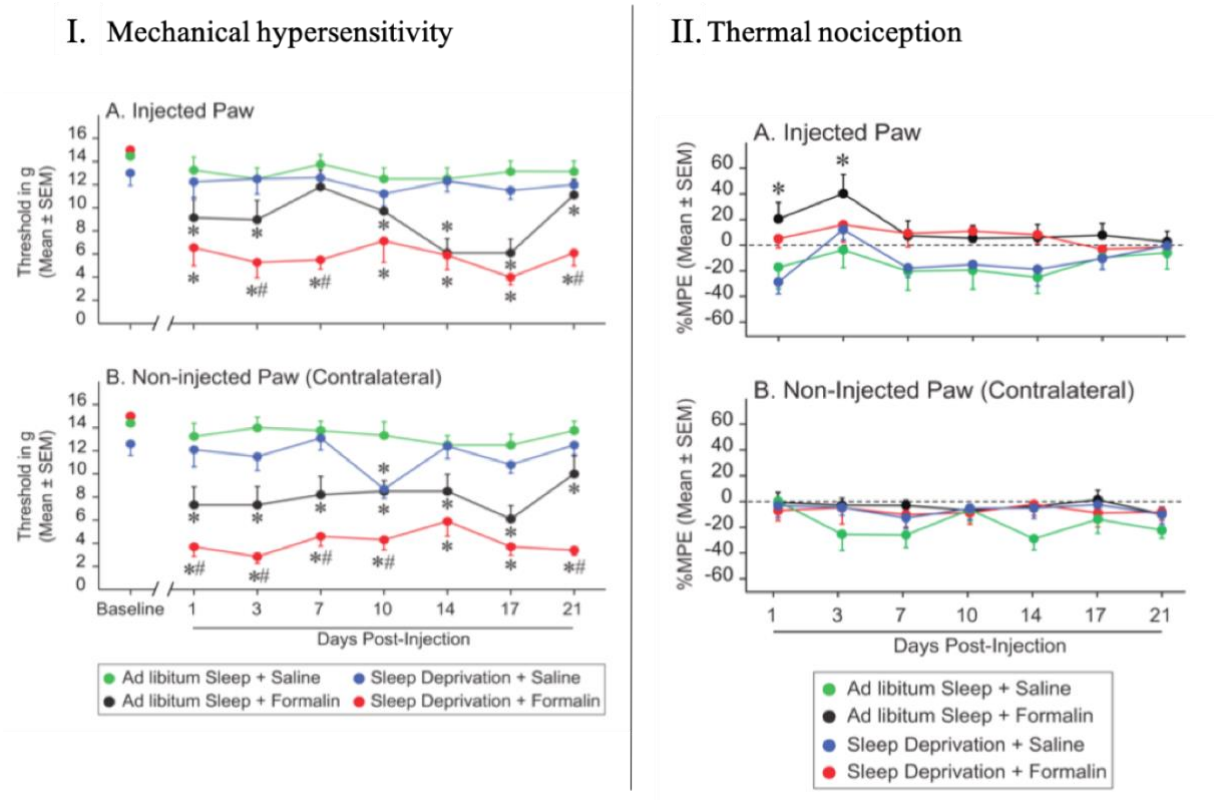

Figure 7: I) Mechanical hypersensitivity assessed during a period of 21 days. The injected paw is compared to the non-injected paw (contralateral). Injection of formalin into hind paw after full sleep and full SD caused persistent bilateral mechanical hypersensitivity. Line breaks indicate the interventions (sleep manipulations and subcutaneous injections), performed in all groups. Asterisks (*) indicate the significant differences compared to the measurements of the baseline. Pound symbols (#) show the significant differences between the groups of *ad libitum* sleep + formalin and sleep deprivation + formalin groups. Bar graphs show mean mechanical threshold for *ad libitum* sleep + formalin and sleep deprivation + formalin groups averaged across the 21-d period post-formalin injection. II). Thermal nociception in injected and non-injected paw during a period of 21 days after formalin injection. The formalin injection caused a significant increase in percentage of maximum possible effect (%MPE) during days 1 and 3 post-injection, compared to *ad libitum* sleep + saline. The baseline (pre-experiment) levels of thermal nociception is indicated with the dotted line at 0% MPE. n = 8 for *ad libitum* sleep + saline, n = 10 for sleep deprivation + saline, n = 10 *ad libitum* sleep + formalin.

Although it seems clear what effect sleep deprivation has on the perception of pain, it has not yet been established what exact mechanism could mediate the process. The next paragraph will be dedicated to highlighting some of the pathways that have proven to be involved in both the processes of pain and sleep.

2.3 Pathways and their relation to sleep and pain

First, it is important to have a better understanding of the pathways that will be explained further on. This is why the general pathway of pain will be explained next.

The pain pathway is characterized by three nerve endings. Nociceptors are the C unmyelinated free nerve endings that convert stimuli into nerve impulses [33,34]. Transduction of the painful stimuli happens in the C unmyelinated nerve endings and the lightly myelinated A δfibers [9,35]. The spinothalamic pain pathway is the pathway that connects the spinal cord to the brain. Information about pain, touch and temperature is communicated through this pathway. Pain is controlled by two regulation pathways, shown in figure 8. The first one, the 'gate control theory' is the afferent regulation. The theory suggests that certain neurons of the dorsal horns are excited by large-diameter sensory axons and also the unmyelinated pain axons. As seen in figure 8A, the projection neuron is both stimulated by the C fiber and Aα or Aβ axons and inhibited by the interneuron. This results in the nociceptive signals transferring to the brain because of the pain axon maximally exciting to the projection neuron. Nociceptive signals can be suppressed by activating the interneuron which is the consequence of the large mechanoreceptive axon firing at the same time. The descending regulation pathway of pain has an important role in the suppression of pain. The periventricular periaqueductal gray matter (PAG) is located in the midbrain, as shown in figure 8B. It consists of neurons and when electrically stimulated, it can cause intense analgesia [9].

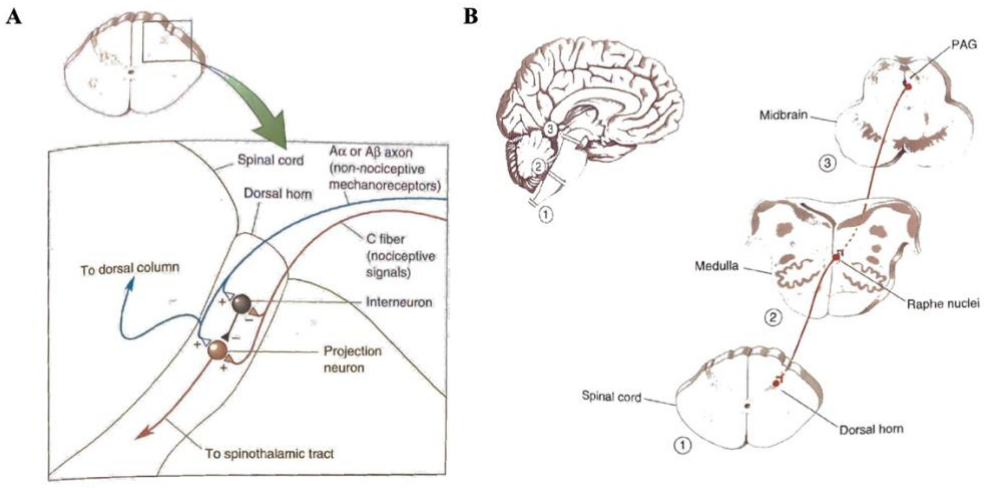

Figure 8: The afferent (A) and descending (B) pain regulation pathways. A) The + signals indicate excitatory synapses, the – signals indicate inhibitory synapses. B) Variety of brain structures indicated with numbers for location in the brain. Adapted from Bear et al [9].

2.3.1 Opioidergic signaling

The first signaling pathway linked to sleep and pain that will be reviewed is the opioidergic signaling pathway. Opioids are a class of drugs that have an effect on the brain and can result in mood changes, drowsiness, mental clouding and nausea [36]. Opioids bind to the opioid receptors in the brain. An example of an opioid drug is endorphin. Endorphins concentrate

specifically in areas that process nociceptive information. When small injections of endorphin are made into the PAG (figure 8B), the raphe nuclei or the dorsal horn, it produces analgesia. The passage of nociceptive signals from the dorsal horn to higher levels of the brain is prevented by the system of endorphin-containing neurons in the spinal cord and brain stem [9]. Opioid receptors have been shown to not only regulate pain, but also sleep. As mentioned earlier, opioid receptors are widely distributed throughout the CNS, specifically in the PAG. The preoptic suprachiasmatic nuclei control the sleep-wake cycles. Moreover, the limbic system and hypothalamus are areas associated with the regulation of pain and sleep in rats [37]. A clinical human study performed by Steinmiller et al, states that a reduction of codeine resulted in an increase in daytime sleepiness which in turn resulted in an increase in complaints of pain [26]. Przewłocka et al deprived rats from sleep using the platform method, as explained earlier. Sleep deprivation was found to alter the μ and δ opioid receptor function in the mesolimbic circuits, which is a dopaminergic pathway. The central opioid receptors are downregulated as a result of sleep deprivation. This downregulation is associated with a decrease in morphine effectiveness, together with a central nervous system opioid insufficiency in the limbic system and hypothalamus [37,38]. This decreased morphine effectiveness could be one of the causes of a lower pain threshold. These results show that the deprivation of REM sleep leads to a change in the opioidergic pathway, which subsequently leads to an increase in the perception of pain.

2.3.2 Dopaminergic signaling

The second pathway is the dopaminergic signaling pathway, also referred to as the mesolimbic pathway or circuit. Dopamine is a neurotransmitter synthesized by the dopaminergic pathway. It has been linked with various diseases such as insomnia, chronic pain and depression [39]. Dopamine can bind to its two receptors, D1 and D2 receptors [40]. In an animal study, Sardi and colleagues used rats who were deprived of sleep to investigate nociceptive behavior. Sleep deprivation was induced using the single-platform method, which eliminates REM sleep and decreases non-REM sleep for 24 hours. Similar to the flower pot technique, whenever muscular atonia occurs in the rats, they will fall off the platform and wake up. The paw-withdrawal method was used to assess mechanical nociceptive behavior. Sardi et al showed that decreased activity at the dopaminergic D2 was present in the nucleus accumbens (NAc), part of the mesolimbic circuit. This could have pronociceptive effects as a consequence from REM sleep disturbance [41]. A study conducted by Taylor and his team states that the stimulation of D2 receptors in the NAc dramatically inhibits the constant perception of pain [42]. This effect could be due to a decreased activity at dopamine D2 receptors in the NAc, possibly caused by a D2 agonist blocking any similar effect. The D2 receptor agonist prevented the pronociceptive effect of REM-SD, while the antagonist had no such effect. These findings indicate that SD increases the pain perception by decreasing the D2 receptors at the NAc. The cause of the decreased D2 receptor activity could be a decreased release of dopamine or a decreased D2 receptor expression. Furthermore, it has been established that in the NAc, dopamine levels are higher during REM sleep and therefore low levels of dopamine are to be expected during REM-SD [41].

2.3.3 GABAergic pathway

Finally, gamma-aminobutyric acid (GABA) has an important role in the processes of both sleep and pain. GABA is an important component of the central nervous system because of its ubiquitous distribution [43]. Glutamate and GABA are neurotransmitters that are involved in the process of both sleep and nociception. Sleep is induced by an increase in hypothalamic GABA level and $GABA_{(A)}$ agonists [44]. GABA binds to the GABA-receptor, which can be either ionotropic $GABA_A$ or metabotropic $GABA_B$. The activation of both of the GABA receptors leads to a decrease in pain perception, thereby inhibiting the spreading of pain impulses [43,45]. A higher level of insular glutamate or reduced GABA levels could contribute to hyper-reactivity of the insula.

The study of Watson et al researched if decreasing the imbalance of the chemicals in the insula would lead to a decrease in neuropathic pain. To investigate this, GABA levels were increased in the insula. Watson et al showed that creating a stimulating imbalance by decreasing levels of GABA was sufficient enough to increase the thermal hyperalgesia and the mechanical allodynia. The animal study of Kamal et al [44] rats were deprived of sleep using the disc-on-water method as used in the study of Hsu et al [48]. Both the control group and sleep deprivation group were placed in the Total Sleep Deprivation (TSD) apparatus for at least seven days. The TSD apparatus consisted of a plastic chamber with a disc to carry the rats, and water underneath. The TSD group was kept awake for 5 days, by forcing physical activity through the disc-on-water method. The animals in the control group were able to rest from 06:00 to 18:00 h. Kamal et al state that total sleep deprivation significantly decreases the GABA concentration in the prefrontal cortex and thus leads to a decrease in pain. Another study investigating the effect of sleep deprivation on GABA was the study of Wang et al [47]. Rats were subjected to sleep deprivation using the flower pot technique for 96 hours. The research was focused on the frontal cortex, hypothalamus and brain stem. The concentration of GABA was raised in the sleep deprived rats, while there were no changes observed in the control group. This would indicate that the sleep deprivation raises the levels of GABA in the brain of the rat, leading to an increase in GABA receptor activation and thereby to a decrease in pain sensation. This is an interesting finding since studies have shown that an elevation in GABA leads to more sleep which would lead to less pain when compared to the clinical and animal studies previous mentioned.

The results of the reviewed studies all confirm that the mentioned pathways have a link to sleep and pain. It is beyond the scope of this thesis to research how these three mechanisms are connected to each other within the brain, since it would not be contributing to answering the main question. That is why the focus is kept on explaining the link that the pathways have to sleep and pain.

3. Discussion

The purpose of this thesis is to investigate what the effect of sleep deprivation is on pain. It has long been established that pain has a negative effect on the sleep of patients with diseases such as FM and RA. More recently it has been discovered that the relationship also works the other way around. Recent studies can help build the hypothesis that it is an ongoing cycle in which patients experience heavy pain and cannot have a full night of sleep. The consequence of this reduction in the quality of sleep is a more painful experience. Several articles have stated that sleep deprivation and pain have an effect on one another [12,13]. It is important to investigate in what way the sleep deprivation influences pain to possibly discover methods to lighten the symptoms of patients.

Overall, the studies reviewed in this thesis support the hypothesis stating that sleep deprivation has a worsening effect on the pain perception, making the subjects more sensitive to pain [27-31]. The results obtained from this literature review build on existing evidence that pain has a lowering effect on sleep and vice versa. While previous research has focused on the effect of pain on sleep, these results demonstrate the lowering effect of sleep deprivation on the pain treshold. However, the formalin test in the Hakki Onen animal study showed no significant difference in the pain related behavior of the rats. Furthermore, the group of Vanini et al showed that, compared to *ad libitum* sleep and a formalin injection, there was no continuing effect on the thermal nociception due to the injection of formalin or sleep deprivation. This is possibly due to the nociceptive behavior assessment not being the most reliable, since rats all possibly have their own way of acting, including in pain. Furthermore, it is possible that formalin has a painful cause for both rested or SD rats, with it resulting in not making a difference between rest or SD. Regarding the limitations of these studies, it could be argued that this decreases the reliability of the results since it is not all in line with the hypothesis.

Since there is a tendency for sleep deprivation to lower the pain threshold, the mechanisms behind sleep and pain have been investigated to create a clearer picture about the link between them. Steinmiller and his team showed that sleep deprivation increases pain by showing a reduction of codeine [26], which in turn resulted in an increase in daytime sleepiness. Furthermore, sleep deprivation of animals resulted in decreased dopaminergic D2 activity which, as a consequence, has pronociceptive effects [41]. Wang et al stated that sleep deprivation results in an increase of GABA and thus an increase in activity for GABA receptors. Subsequently, the pain perception is increased [47]. Yet, one study contradicts these finding by stating that sleep deprivation results in a lowering of the GABA levels in the prefrontal cortex resulting in a lower pain perception [44]. It is interesting to note that sleep increases as a result of the elevation of hypothalamic GABA levels and $GABA_A$ agonists. The finding of Wang et al is not in line with this finding, since sleep deprivation should be lowering the levels of GABA. The study of Kamal et al agrees with this statement, claiming that sleep deprivation lead to a decrease in the concentration of GABA in the prefrontal cortex. There is a possibility that after a longer period of time the GABA levels increase or decrease at certain parts of the brain, which would explain the contradicting findings as

described above. The mechanism behind this process has not been established yet. Due to the studies conducting their experiments in different areas in the brain, it is hard to draw a conclusion about the effect of sleep deprivation on GABA levels focusing on one area. It is highly possible that the GABA levels are raised in one part of the brain after lack of sleep and decreased in another part. Furthermore, it is possible that an increase in GABA could result in a higher perception of pain while in another part of the brain it results in a lower perception. That is why the recommendation for future research is to focus on one part of the brain, or to focus on extending the sleep deprivation period.

Then, the generalizability of the results is limited by the differences of depriving the subjects of sleep and characterizing it in different ways. The extent to which sleep deprivation was used is different with every study. The participants in the human study of Hakki Onen et al were either SWS- or REM-sleep deprived [28]. The clinical study of both Schuh hofer et al and Krause et al deprived the subjects of sleep completely [27,29]. The animal study of Hakki Onen et al used the flower pot technique to REM deprive the rats of sleep [30, 31]. Vanini et al deprived the rats of sleep completely by stimulating the rats by using sound or touch. For the characterization of sleep, studies used sleep waves while others relied on the physical characterization of sleep. An important point of discussion is if a similar amount of sleep time is deprived in all studies. If not, it becomes harder to compare resulting in a decrease in liability.

The conclusion could also be argued by the pain threshold which differs from person to person and possibly also for animals. The question raises what effect this has on the reliability of the results. People and possibly animals could have a different interpretation of what pain feels like. It is possible that some humans have a lower pain threshold than others. Besides that, humans are able to communicate their pain very specifically verbally while with experiments on animals the assessment of pain relies on their nociceptive behavior and emitted sounds. For both the human studies of Schuh-Hofer et al [29] and Krause et al [27] Quantitative Sensory Testing (QST) was used. QST is a method to assess the sensory nerve function, in which one stimulus can be studied at once [49]. In the case of Schuh-hofer et al, cold, warm and mechanical stimuli were tested while Krause et al used the warmth QST. In the clinical study of Hakki Onen et al [28], the heat pain tolerance threshold (HPTT) and pressure pain tolerance threshold (PPTT) test were conducted on the subjects. For the animal study of Hakki Onen et al [30], different methods were used to assess the nociception. The animal study of Vanini et al [31] used the von Frey test to evaluate mechanical sensitivity. Since the studies described above all use different types of instruments and methods to assess the amount of pain felt by humans and rats, the results could be perceived as less reliable as when the same methods would be used. It is hard to say which method is more reliable, since it depends on the pain perception of the subjects and animals.

Finally, in the field of sleep deprivation and pain it is important to continue both human and clinical studies, since the ethics of an experiment are limited for clinical studies and the communication for animal studies. A longer period of sleep deprivation would only be possible in animal studies, reason being that it is unethical to deprive humans of sleep for a

longer period of time. As mentioned before, animals are not able to communicate their amount or their type of pain and humans are. This makes the clinical experiments essential for research. Furthermore, in clinical trials researchers could be focusing on descent or age, and if this has any effect on the cycle of sleep deprivation and pain. The field cannot work without either clinical or animal studies.

Further research is needed to establish and strengthen the hypothesis that sleep deprivation has a lowering effect on the pain threshold. Moreover, research should be conducted about the process behind pain and sleep, since there is not enough known about this field and the results contradict each other. It should be focussed on the different areas in the brain and how neurotransmitters work locally. It is very possible that certain neurotransmitters could be increasing pain in one area and reducing it in another. Secondly, it is of interest to wage in important factors as age, sex, weight, descent and others in human studies. It is possible that as humans age, their pain perception gets worse, making them more sensitive to pain, and this should be taken into account. Studies should be focused on one of the factors at the same time to get a better grasp at what is worsening the vicious cycle of pain and sleep deprivation. Additionally, it would be of interest to research the long-term effects of sleep deprivation. Since it is ethically not possible to perform such experiments on humans, it would be more appropriate to assess this in animals.

Both pain and sleep remain complicated systems in which research is essential. As a result, it will contribute to lightening the symptoms of patients, who will be able to have a full night of sleep again.

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