Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Kavli Institute for Systems Neuroscience

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Sleep and Pain

An EEG study of how sleep affects pain perception

Master's thesis in Neuroscience
Supervisor: Dagfinn Matre & Petter Moe Omland
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Abstract

Background: Experimental studies show that lack of sleep is associated with altered pain perception. Studies using brain imaging techniques are needed to further the development of objective neurophysiological correlates of pain.

Objective: This thesis investigates how restricted sleep affects perceived pain intensity and electrical brain responses during experimental pain stimulation.

Methods: A within-subject cross-over design was used on a sample of Norwegian shift workers in order to compare perceived pain intensity after two nights of habitual sleep, with perceived pain intensity after two nights of restricted sleep. Pain was induced by a 2-min contact-heat stimulation to the skin. Pain intensity was rated on a visual analogue scale (VAS). Brain responses were recorded with electroencephalogram (EEG) through a 32-electrode cap. Dynamic spectral analysis was performed on the EEG signals by the Continuous Wavelet Transform. Statistical analysis was performed by Linear Mixed Models. The EEG activity was analyzed in terms of the delta, theta, alpha, beta and gamma frequency ranges. Both the mean level of activity (static EEG indices) and the dynamics of the activity (dynamic EEG indices) were investigated.

Results: Perceived pain intensity was significantly higher after restricted sleep as compared to habitual sleep. Pain intensity increased over the 2-min period. No significant sleep-dependent changes were found in any of the static EEG indices. The dynamic EEG indices showed that increased alpha activity was associated with increased pain scores after habitual sleep. Contrary, after restricted sleep, decreased alpha activity and decreased theta activity was associated with increased pain scores.

Conclusion: The findings in this thesis strengthen the notion that sleep loss leads to an increase in perceived pain intensity. Brain mechanisms underlying the hyperalgesic effect of restricted sleep may involve alpha and theta activity.

Keywords: hyperalgesia, restricted sleep, pain intensity, EEG, alpha band, theta band, night shift work

Sammendrag

Bakgrunn: Eksperimentelle studier har vist at søvnmangel er assosiert med endringer i smertepersepsjon. Flere hjerneavbildningsstudier trengs for å utvikle objektive nevrofysiologiske korrelater til smerte.

Hensikt: Denne studien undersøker hvordan søvnmangel påvirker opplevd smerteintensitet og elektrisk hjerneaktivitet under eksperimentell smertestimulering.

Metode: Et innen-gruppe design ble brukt på et utvalg norske skiftarbeidere for å sammenligne opplevd smerteintensitet etter to netter med normalsøvn og to netter med søvnmangel. Smerteintensitet ble vurdert med en visuell analog skala (VAS). Elektrisk hjerneaktivitet ble målt med elektroencefalogram (EEG) gjennom en elektrodehette med 32 elektroder. Dynamisk spektralanalyse ble gjennomført på EEG signalene med Kontinuerlig Wavelet-Transformasjon. Statistisk analyse ble gjennomført med Mixed Models. EEG aktiviteten ble delt inn i de fem frekvensbåndene delta, theta, alpha, beta og gamma og analysert deretter. Både gjennomsnittlig aktivitetsnivå (static EEG indices) og dynamisk endring i aktiviteten (dynamic EEG indices) ble undersøkt.

Resultater: Opplevd smerteintensitet var signifikant høyere etter to netter med søvnmangel enn etter to netter med normalsøvn. Opplevd smerteintensitet økte over de 2 minuttene med smertestimulering. Ingen signifikante endringer ble observert i de statiske EEG analysene. De dynamiske EEG analysene viste at økning i alfa-bånd aktivitet var assosiert med økning i smerteskårer etter normalsøvn. Etter søvnmangel var reduksjon i alfa-bånd aktivitet og theta-bånd aktivitet assosiert med økning i smerteskårer.

Konklusjon: Resultatene støtter tidligere funn om økt smerteintensitet etter søvnmangel. Aktivitet innen alfa-båndet og theta-båndet kan være assosiert med mekanismene som understøtter sammenhengen mellom søvn og smerte.

Nøkkelord: hyperalgesi, smerteintensitet, søvnmangel, EEG, alfa aktivitet, theta aktivitet, nattarbeid

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I would like to express my gratitude to my main supervisor Dagfinn Matre (PhD) for his guidance and support. Thank you for all the helpful feedback and critical questions along the way. I have appreciated the opportunity to learn from your extensive knowledge on pain and sleep. I would also like to extend my gratitude to my supervisor Petter Moe Omland (M.D., PhD). Thank you for taking time in-between patients to teach me about artifact removal and for providing thorough feedback on my thesis. Thank you also to Tone Opdahl Mo (PhD) for reading through my work and providing me with a helpful outside perspective.

A special thanks to my family for helping me keep my spirits high, and to my peers for initiating "stretching hour" to make sure we all got out of the office from time to time. Last but not least, I would like to thank my partner Philip for his invaluable emotional support and countless efforts to make sure I drink enough water.

Preface

This thesis is based on data from a research project at the National Institute of Occupational Health, Norway. The research group is led by Dagfinn Matre and specializes in the health effects of shift work. I have been given access to raw data form one of their experimental studies regarding sleep restriction and pain.

This thesis is written in the reference style of APA (7th edition). Some adjustments have been made in order to improve the readability. The implemented changes are in accordance with NTNU guidelines for universal design.

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Introduction

This thesis aims to further our understanding of how sleep affects pain perception by studying patterns of activity in the brain. Research has shown that chronic pain can reduce the quality of life significantly for those affected (Andersen et al., 2018). Furthermore, chronic pain and musculoskeletal pain is an increasing concern for both healthcare and social security systems (Vos et al., 2017). It has also become the most common cause of disability world-wide (Mills et al., 2019). Musculoskeletal diseases and other pain related issues are one of the most common causes for sick leave in the work force, and can in some cases lead to early retirement (Bergman et al., 2001). Studies show that chronic musculoskeletal pain is a common complaint in the general population, and especially widespread in the industrialized world (Bergman et al., 2001). European studies estimate that chronic pain affects 19% of the adult population in the European Union (EU) (Breivik et al., 2006). Similarly, data from The Trøndelag Health Study (HUNT) indicates that as much as 30% of Norwegians struggle with chronic pain (Landmark et al., 2018). Additionally, a report by the Global Burden of Disease project (GBD), lists lower back pain as one of the health issues that could potentially lead to a reversal of the global health gains seen over the last century (Vos et al., 2017). With an aging population, one can only expect to see more of pain related issues as the prevalence of disabling diseases generally increase with age (Vos et al., 2017).

What is causing this "epidemic of pain"? A contributing factor may be the consequences of disturbed sleep. Haack et al. (2009) found that voluntary sleep loss led to increased pain sensitivity in healthy research participants, and that these subjects reported more instances of spontaneous pain than the control group. A growing body of research supports this proposed link between sleep loss and complaints of pain (Kaila-Kangas et al., 2006; Uhlig et al., 2018). Additionally, multiple studies indicate that shift work is a risk factor for developing sleep problems (Kecklund & Axelsson, 2016). Furthermore, shift work has been linked with musculoskeletal injury (Trinkoff et al., 2006) and other pain disorders, such as lower back pain (Zhao et al., 2012). As a result of these and other findings, pain related issues are increasingly being understood in the context of sleep (Uhlig et al., 2018). The mechanisms underlying this connection are, however, still not well understood. Some put forward models of inflammation, others hint at negative affect and depression as possible explanations (Babiloni, A. H. et al., 2020). A third direction focuses on how pain and sleep affect the patterns of activity in the brain. This is where the focus of this thesis lies.

This thesis will investigate how restricted sleep affects perceived pain intensity and electrical brain responses during experimental pain stimulation. First, I will define pain and

describe the pathway of which pain is transmitted. Second, I will present the sleep cycle, consequences of sleep loss, and potential mechanisms supporting the relationship between sleep and pain. Third, I will present the brain imaging method EEG and how it can be used to study pain perception, in addition to summarizing some relevant findings on EEG brain activity related to sleep and pain. Following this, the aim, objectives and hypotheses of this thesis will be accounted for. Subsequently, the methods that have been used to study perceived pain intensity and electrical brain responses will be described in detail. Lastly, the results are presented and discussed in the context of the presented empirical findings from the field of pain research.

Understanding pain

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential damage, or described in terms of such damage" (IASP Task Force, 2012).Though most pain will subside over time, a considerable amount of people struggle with chronic pain. The IASP defines chronic pain as "pain lasting for more than three months" (Nicholas et al., 2019). Chronic pain can be understood as the result of ongoing damage that prevail without successful healing. However, pain can also persist with no apparent cause after the initial healing of the damaged tissue. As for what causes this pathological process, researchers recognize that multiple mechanisms might be at play (Landmark et al., 2018). Some suggest a neurochemical imbalance in the central nervous system, others propose that malfunctions in the descending pain modulation system might be to blame (Arnold et al., 2016). The list of possible explanations goes on, and includes factors such as a decrease in endogenous substances, like melatonin and dopamine, and the impact of cognitive states, like negative affect and depression (Babiloni, A. H. et al., 2020).

To study the causes and effects of pain, one must find a way to measure it. The field of pain research often relies on the use of pain scales. Common examples are the visual analogue scale (VAS) and the numeric rating scale (NRS) (Haefeli & Elfering, 2006). Scales like these are used for patients and volunteers in pain experiments to rate the intensity of their pain. The end points of the scales are defined at the opposite ends of the spectrum. Commonly a scale will range from 0-10, with endpoints of 0="no pain", and 10="worst imaginable pain". Though self-reported scales are one of the most common ways to measure pain, it is a highly subjective measure (Xu & Huang, 2020). Moreover, identical pain stimuli are often

experienced differently between individuals, and each individual can exhibit different responses to the same pain stimulus at different points in time (Schulz et al., 2011).

A lot of work has been done to find more objective measures of pain (Gatchel et al., 2007). Combining different measuring techniques has the potential to further our knowledge of the biological, psychological and social aspects of pain (Morton et al., 2016). Approaches such as subjective ratings of pain, or information on how family and friends react to the patient's pain experiences, can lead to a better understanding of the psychological and social mechanisms involved (Gatchel et al., 2007). The biological processes are better understood in terms of neurophysiological recordings of the neuronal activity in the pain pathway (dos Santos et al., 2016). An approach that is especially relevant for this thesis, is the hunt for neurophysiological correlates of pain through the use of brain imaging techniques. Combined, different techniques can help bridge the gap between our subjective experience of pain and the biological processes that are supporting it (Morton et al., 2016).

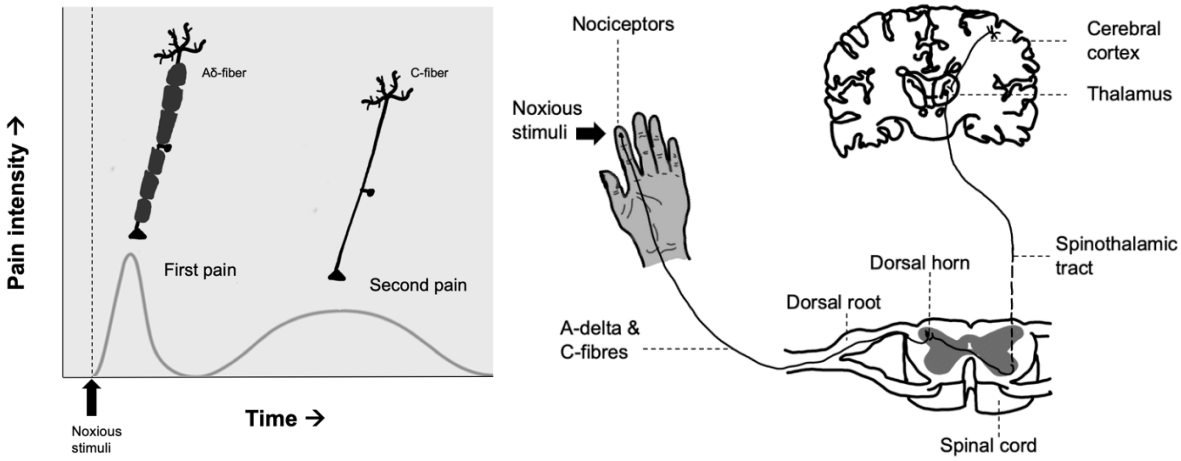
The pain pathway

When working with the phenomenon of pain, one must distinguish between pain and nociception. Pain embodies both the sensory and cognitive components of pain, as well as our emotional response (Melzack & Casey, 1968). Nociception strictly refers to the process of encoding noxious stimuli, i.e. the very biological response of the sensory nervous system as it comes in contact with harmful, or potentially harmful, stimuli (Ploner et al., 2017). To further understand the pathways that lead to pain perception, it is useful to know how the transduction, transmission and modulation of pain takes place.

Transduction occurs in the periphery at the location of the pain, i.e. the nociceptive stimulus. We have three different types of nociceptors; (1) thermoreceptors that are activated by temperatures above or below certain thresholds, (2) mechanoreceptors that respond to mechanical pressure or distortion, and (3) polymodal nociceptors which can respond to both mechanical stimuli and temperature, as well as to chemicals released by tissue damage and inflammation (Basbaum & Jessell, 2013). Nociceptors like these can be found both externally, e.g. in the skin, and internally, e.g. in muscles, joints and organs etc. When nociceptors are activated by noxious stimuli, they translate the noxious events into chemical events and initiate signal transmission. The signals are sent from the periphery to the central nervous system (CNS) along a neuronal pathway that goes from the nociceptors, via the dorsal horn of the spinal cord, and then further up to the thalamus (Figure 1).

The first step of this cascade, the neuronal path from the nociceptors to the dorsal horn, gives rise to the concept of first and second pain. First pain is the result of noxious stimuli that is transmitted via fast myelinated A-δ fibers (33-75 m/s). This pain is perceived as brief and well localized. Second pain is transmitted at a slower rate through unmyelinated c-fibers (0.5-2 m/s). This pain lasts longer than first pain, is less well localized, and can induce a burning, dull sensation (Basbaum et al., 2009). Regardless of the type of fiber that transmits the pain from the nociceptors to the dorsal horn, they enter the spinal cord via the dorsal roots. Once they reach the dorsal horn, they form synapses with projection neurons in lamina I and II. From here, the projection neurons cross the midline and ascend up to the brainstem and thalamus. This part of the pain pathway is known as the spinothalamic tract, and is the major ascending pathway of pain and temperature (Basbaum & Jessell, 2013). Third-order neurons localized in the thalamus project the signals further, to the somatosensory cortex and other brain areas.

Figure 1



Note. Left: Transmission of nociceptive stimuli through myelinated A-δ fibers and unmyelinated C-fibers. Right: The pain pathway.

Both the ascending path of pain transmission, as well as the descending modulatory pathway play a role in how pain is perceived. Pain modulation can occur in multiple ways; in the synaptic junctions in the ascending pain pathway, in the descending pathway, through hormonal or cortical activity, or in the periphery at the source of the pain. The actions taken by the CNS to modulation pain is referred to as endogenous pain modulation (EPM) and can both reduce or augment the incoming nociceptive signals (Yarnitsky, 2015). Multiple mechanisms and processes can trigger pain modulation, and the interactions and implications

of these are still being explored by the scientific community, see for example Damien et al. (2018) for a review on pain modulation.

Theories of pain

Theories of pain continue to evolve as we expand our knowledge of the various aspects of pain. Although much has been discovered regarding the first order A-δ- and C-fibers in the ascending pathway, much less is known when it comes to the second and third order neurons (Moayedi & Davis, 2013). Advances in fields such as neuroimaging and molecular medicine help us reject and renew our working hypotheses. In this section, I will briefly present one older model of great significance to the development of pain research, as well as two pain models that have been developed more recently.

Melzack and Wall presented the Gate Control Theory in 1965, as an attempt to bridge the gap between other existing theories of the time. Melzack and Wall claimed that the fibers of the nociceptors synapsed in in three different regions of the dorsal horn; the substantia gelatinosa, the dorsal column, and with of group of cells they named transmission cells (Gatchel et al., 2007). The substantia gelatinosa of the dorsal horn was said to be the "gate", modulating the transmission of sensory information from the nociceptors to the transmission cells. Interneurons would facilitate the opening and closing of the "gate", depending on whether the nociceptive information exceeded the inhibitory activity. The gate control theory led to a spur of studies but has later been proved to be somewhat inaccurate. Amongst other things, statements regarding the neuronal architecture of the spinal cord were flawed, and the modulatory system they described does not match with newer findings on descending projections from the brain stem (Chen, 2011).

The idea of the pain matrix offers an alternative way to view pain. There is no single area in the brain solely responsible for pain processing. Instead, pain is seen as the consequence of activation in a widely distributed neural network. The pain matrix consists of areas that have proved to consistently respond to pain when studied with various brain imaging techniques. This includes areas such as the primary and secondary somatosensory cortex, the anterior cingulate cortex, the thalamus, the insular cortex and the prefrontal cortex (Morton et al., 2016). These areas are not only activated by pain, but also by other sensory, motor and cognitive functions. The pattern and distribution of activity within the pain matrix is largely dependent on the type of pain induced, as well as emotional and cognitive aspects, e.g. expectations and attention (Morton et al., 2016).

A second model, the biopsychosocial model of pain, is in line with this view on pain as a multifaceted process. In this model, pain is conceptualized as a result of the interplay between biological, psychological, and social factors (Hanssen et al., 2017). The biological part deals with the biochemical process of nociception. The psychological aspect encompasses both our emotional and cognitive responses. Our emotional responses are considered to be the most immediate reactions, while our cognitions make meaning of our pain experiences (Gatchel et al., 2007). Social factors include environmental stressors, social support and previous treatment experiences etc. (Gatchel et al., 2007).

The importance of sleep

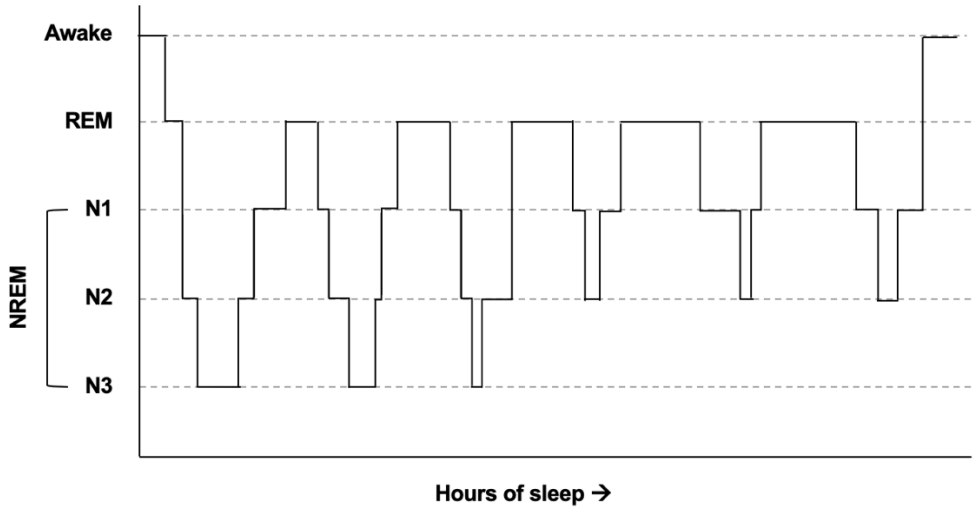
Multiple studies have shown that pain and sleep is interconnected (Finan et al., 2013). In order to understand chronic pain, there is a need to understand sleep. In this section I will give an overview of the sleep cycle and the consequences of sleep loss in an attempt to better understand the link between pain and sleep.

As humans, we spend approximately one-third of our lives sleeping. Sleep is commonly divided into two distinct phases; REM (rapid eye movement) and non-REM sleep (NREM) (Figure 2). REM-sleep is characterized by an inhibition of the motor neurons in the descending path of the spinal cord, resulting in an almost complete lack of movement from the neck and down (Kandel, Schwartz & Jesell, 2013). NREM is divided into three different stages, N1, N2 and N3. N1 is described in terms of drowsiness and the occurrence of low amplitude theta waves (Acharya et al., 2005). In N2, eye movements stop, and slower brain waves become apparent. Moreover, this stage is characterized by distinctive brain waves called sleep spindles and K-complexes (Acharya et al., 2005). Following these stages of lighter sleep comes the stage of deep sleep, N3, formerly known as N3 and N4. Deep sleep is characterized by slow wave activity, usually in the 0.5 to 4 Hz area (Saper et al., 2010). This is the stage where it is most difficult to wake someone from their sleep.

During a normal night of sleep, a healthy subject will go from light sleep to deep sleep to REM-sleep, before cycling back to light sleep again. A full NREM to REM sleep cycle lasts approximately 90 min, and a typical night of sleep consists of 3-5 sleep cycles (Babiloni, A. H. et al., 2020). As each cycle is completed, the duration of the next REM sleep phase becomes longer, the amount of deep sleep is reduced, and N2 becomes more prominent (Saper et al., 2010).

Figure 2

A typical night of sleep



Although there is consensus on the importance of sleep, there are multiple theories as to why sleep is so crucial. Porkka-Heiskanen (2013) presents three main theories; a metabolic model, a synaptic model, and an immunological model. In short, the metabolic model states that sleep allows for restoration after energy depletion (Benington & Heller, 1995), the synaptic model claims that neural plasticity is maintained and regulated during sleep (Tononi & Cirelli, 2006), and the immunological model proposes that sleep plays a role in maintenance of certain functions of the immune system (Krueger et al., 2011).

In other words, getting enough quality sleep is important, both to maintain good health and to uphold daytime functioning. Unfortunately, there are many individuals who experience sleep related issues. Insomnia is reported to be the highest occurring sleep complaint in most populations (Uhlig et al., 2014). Individuals suffering from insomnia have a difficulty falling asleep or staying asleep. As many as 30% of the adult population are troubled by insomniac symptoms, and about 10% of these meet the diagnostic criteria for insomnia (Morin et al., 2009). Other common sleep disorders are obstructive sleep apnea, characterized by repeated episodes of cessation of breathing during sleep, and restless leg syndrome, where a prickling or burning sensation can be felt in the leg during rest and sleepiness, urging the individual to move (Sateia, 2014).

Multiple studies show that these disorders and others sleep issues can lead to severe negative consequences (Krause et al., 2019; Orzeł-Gryglewska, 2010;Zhang et al., 2019). Some adverse effects include alterations in the regulatory functions of neuronal and endocrine

systems. This can further affect cognitive functions, such as memory and concentration, as well as result in emotional symptoms (Orzeł-Gryglewska, 2010). Furthermore, sleep related problems are a risk factor for developing pain disorders, e.g. chronic musculoskeletal pain (Mork & Nilsen, 2012) and headache (Ødegård et al., 2010). Additionally, sleep issues are repeatedly correlated with a heightened acute pain perception and more instances of spontaneous pain (Haack et al., 2012; Kundermann et al., 2004; Lentz et al., 1999). Experimental sleep restrictions have also been shown to lead to increased pain sensitivity (Matre et al., 2015, 2017).

Animal studies find a consistent effect of REM sleep deprivation on pain perception, but there is a lack of studies on NREM sleep deprivation (Lautenbacher et al., 2006). Human studies have more inconsistent findings regarding REM sleep deprivation, but generally agree on the hyperalgesic effect of sleep deprivation. Hyperalgesia refers to the phenomenon where normally painful stimuli elicit pain of grater intensity (Basbaum et al., 2009). Although a lot of evidence indicates that pain perception is affected by sleep deprivation, more research is needed in order to draw a firm conclusion on whether it is general sleep deprivation that leads to hyperalgesia, or rather the disruption of specific sleep stages (Lautenbacher et al., 2006).

Interactions between pain and sleep

Many studies have found correlations between sleep disturbances and pain. There is however still a discussion regarding the directionality of the association between the two. Whereas some propose that sleep disturbances affect pain perception, others find that pain can lead to sleep loss and reduced quality of sleep (Babiloni, A. H. et al., 2020). A recent review article highlights that the evidence leans towards the former, namely a consistent unidirectionality in which sleep predicts next-day pain (Andersen et al., 2018). This unidirectional effect is especially strong in experimental and acute pain models. Most of the data does however deal with the association between insomnia and pain. More longitudinal studies and studies on a larger variety of sleep disturbances are needed to strengthen the claim of the unidirectional effect of sleep on pain.

While the sleep-pain relationship is firmly established, the potential mechanisms that underlie the interaction are currently not well understood. As mentioned earlier, some point towards the role of inflammatory markers. Alterations in sleep are reported to lead to alterations in immune responses, potentially aggravating chronic pain disorders (Babiloni, A. H. et al., 2020). Others are considering the implications of affect and mood. Disrupted sleep can lead to mood disturbances, and negative affect can lead to sleep disturbances (Konjarski

et al., 2018). Furthermore, higher levels of negative affect is thought to increase hypervigilance to pain (Babiloni, A. H. et al., 2020). This can cause a sensitizing of pain to take place. Positive affect on the other hand, can increase the resilience of the individual, and attenuate both negative responses to pain and the overall perception of pain (Finan et al., 2013; Hanssen et al., 2017).

Alterations in endogenous pain modulation is considered as another possible explanation. Alterations or deficiencies in endogenous pain modulation can result in both increased facilitation and inhibition of pain (Babiloni, A. H. et al., 2020). Insufficient sleep over a prolonged period of time can impair inhibitory functions of endogenous pain modulation, and in turn increase vulnerability to pain (Haack et al., 2012). One way this could impact pain is through temporal summation, i.e. the phenomenon where continuous or repeated pain can evoke an increasingly intense sensation of pain (Vierck et al., 1997). A study by Staud et al. (2003) found that chronic pain patients, when compared to pain-free controls, had enhanced temporal summation. Another experimental sleep study observed that sleep deprivation decreases temporal summation (Haack et al., 2012). Improving our understanding of how electrical brain activity responds to pain stimuli after sleep loss could lead to insights regarding the effect of sleep on endogenous pain modulation.

Electroencephalography and brain oscillations

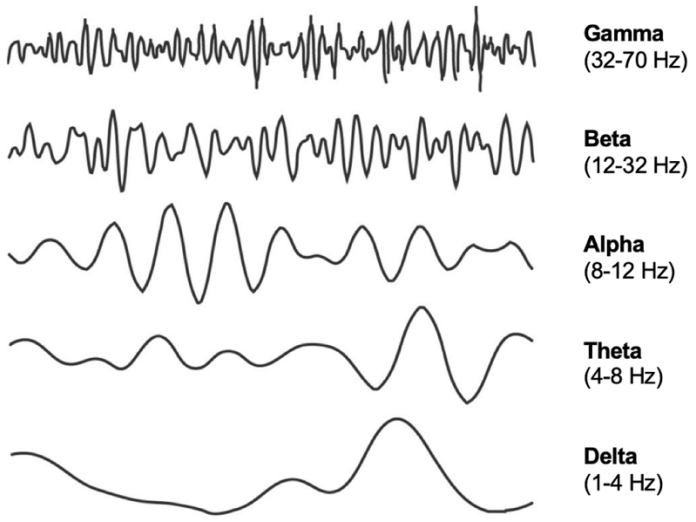
Studying the electrical activity of the cortex can help advance pain research, as cortical activity is implicated in both pain perception and modulation. In recent years, electroencephalography (EEG) has been widely used as a technique to measure alterations in brain activity related to pain perception (Xu & Huang, 2020). EEG is a non-invasive brain imaging technique that allows you to study brain activity down to the level of milliseconds. A set of small electrodes are place on the scalp, and the electrodes record the electrical activity of the brain. The activity that is recorded with EEG stem from the post synaptic potentials of cerebral cortical neurons near the scalp. Each EEG electrode records the sum of synchronized voltage fluctuations in the surrounding area, and the fluctuations arise as a result of ionic currents within the neurons (Louis & Frey, 2016). Networks of neurons synchronize and form overarching patterns of electrical activity. These patterns are termed oscillations and represent the summation of inhibitory and excitatory post synaptic potentials generated by thousands of nearby neurons (Louis & Frey, 2016). The oscillations do not arise as a simple product of summation, the neuronal activity is also regulated by interneurons. Interneurons form connections between different cerebral cortical neurons, as well as between cortical and

subcortical neurons. This develops feed-back links that can support the synchronization and desynchronization of large neuronal networks (Louis & Frey, 2016).

Patterns of synchronized neuronal activity can be analyzed with EEG. In humans, the oscillatory activity is commonly divided into five main frequency bands; delta, theta, alpha, beta, and gamma (Figure 3). Researchers vary in how they define these bands in terms of frequency range (Babiloni, C. et al., 2020). In this thesis, the following subdivision is used; delta (<4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-32), and gamma (32-70 Hz) (Gram et al., 2015). The reader is referred to (Babiloni, C. et al., 2020)for the International Federation of Clinical Neurophysiology's latest recommendations regarding the subdivision of scalp recorded resting EEG rhythms. The five oscillatory rhythms can be divided into two main categories. The delta, theta and alpha rhythms can be referred to as global processing modes, and are found to span large parts of the brain (Knyazev, 2012). The beta and gamma rhythms are often distributed over a more limited topographical area of the brain (Knyazev, 2012). The three global oscillatory rhythms are hypothesized to facilitate integration across the cortex., while the high frequency beta and gamma rhythms are postulated to be involved in coordination of fast and specific cognitive processes that operate within tens of milliseconds (Knyazev, 2012).

Figure 3

Characteristics of the five EEG frequency bands in humans (Nowack, 1995)



The activity within the five frequency bands change according to variations in stimuli, as well as with changes in cognitive and behavioral processes. Enhanced synchronization between populations of neurons leads to an increase in power within a given frequency band,

while desynchronization of neural activity leads to a decrease in power. A change in power within one frequency band is likely to be interconnected with the activity within other bands, as the slower oscillatory rhythms are thought to modulate the faster ones (Knyazev, 2012).

A lot of work has been done in an effort to uncover the functional contributions of the different brain rhythms. In this section I will summarize some of the general findings, before turning the focus back to pain research. The delta rhythm is most prominent in early developmental stages and during deep sleep, though it has also been implicated in processes of motivation, such as hunger and sexual arousal (Knyazev, 2012). The theta rhythm on the other hand, is indicated to be important for learning and memory processes, as well as spatial orientation (Kahana, 2006). Findings regarding the theta rhythm mostly stem from animal research on rodents, but bursts of theta activity have also been observed in humans during spatial orientation tasks (Kahana, 2006). The results reported from human studies are however more ambiguous than those involving rodents (Kahana, 2006). The alpha rhythm has been implicated in memory processes, more specifically in memory retrieval and attention related tasks (Klimesch, 1997). The activity within the beta band is less explored. A review by Engel and Fries (2010) summarizes the current finding on the beta rhythm and puts forward a hypothesis of maintenance of current sensorimotor events and cognitive states. Lastly, the gamma band has been linked with a range of functions. Amongst them are; attention, multisensory integration and preparation of movement, moreover, it has even been suggested tied to conscious awareness (Engel & Fries, 2010). Although the aforementioned brain rhythms have been associated with a wide array of different cognitive and behavioral correlates, a lot still remains to be discovered (Kahana, 2006). The results from this thesis may contribute to our understanding of how the different brain rhythms are implicated in pain perception.

Event related potentials and continuous EEG

While most researchers who use of EEG to study pain measure event-related potentials (ERPs), this thesis will analyze continuous EEG recordings. ERPs are fast electrical changes occurring in the brain phase-locked in response to a specific event induced by the experimenter. The experimenter can induce acute pain in the study subject while simultaneously recording the EEG activity. A short segment (a few seconds) of the EEG data can then be used to analyze changes in activity that is directly related to the experimental pain-event. This is a useful model to learn more about pain. However, a different approach can be taken by analyzing continuous EEG recordings of longer lasting pain stimuli. Some

researchers deem this approach a better fit to study chronic pain than ERPs, as it allows for an exploration of EEG activity patters that might only become visible over a longer period of time (Nir et al., 2010).

Electrophysiological markers of pain and sleep loss

With reliable electrophysiological correlates of pain, we can learn more about how pain is transmitted and modulated (Tracey, 2008). This knowledge could facilitate the development of more objective measures of pain and thus enable us to find better and more effective ways to detect and treat chronic pain diseases (Xu & Huang, 2020). Recent review articles highlight several EEG activity patterns associated with pain perception (dos Santos et al., 2016; Peng & Tang, 2015; van der Miesen et al., 2019; Xu & Huang, 2020). Amongst them, multiple studies report increased activity in the low range of the theta frequency band in patients suffering of neurogenic chronic pain (Llinás et al., 1999; Sarnthein et al., 2006; Stern et al., 2006). However, in an experimental study with healthy subjects, a decrease in theta activity was observed in relation to an increase in pain (Gram et al., 2015).

Another commonly reported finding is the correlation between alpha activity and subjectively rated pain scores (e.g. Nir et al., 2010). This indicates that changes in alpha activity could reflect changes in pain intensity. In an experimental study with healthy subjects, a decrease in alpha activity was seen in response to a pain stimulus designed to mimic chronic pain (Gram et al., 2015). In addition, multiple studies report a decreased level of alpha activity in individuals experiencing chronic pain when they are compared with healthy controls (Camfferman et al., 2017; Jensen et al., 2013). Camfferman et al. (2017) suggest that findings regarding the theta and alpha band could be a marker of abnormal low frequency oscillations between the thalamus and the cortex. Both the thalamus and the cortex are part of the pain pathway, and abnormal signaling between the two could be involved in the observed changes in pain perception.

A third frequency band implicated in the experience of pain is the beta frequency band. An increase in activity in the lower range of the beta frequency band has been observed in patients with chronic pain when compared to pain-free subjects (Stern et al., 2006). Similarly, increased activity in the higher frequencies of the beta band was observed in an experimental pain study with healthy subjects (Gram et al., 2015). Additionally, Gram et al. (2015) present findings which suggests that increased activity in the gamma band could be related to chronic pain (Gram et al., 2015).

EEG has also been used to study the effects of sleep loss. A decrease in alpha activity was seen in resting EEG recordings from individuals who experienced a 24-hour sleep deprivation (Kim et al., 2001). A similar decrease in alpha activity was found in a study where subjects stayed awake for 40 hours (Strijkstra et al., 2003). Furthermore, Strijkstra et al. (2003) report an increase in theta activity following sleep deprivation.

Aims, objectives and hypotheses

The aim of this thesis is to further our understanding of how sleep affects pain perception by studying patterns of activity in the brain. The research objectives are:

- to examine whether perceived pain intensity in response to heat is increased after restricted sleep, compared to after habitual sleep.
- to identify any patterns in the EEG activity that can be associated with changes in perceived pain intensity.

These research objectives will be approached in two ways. First, analyses of static data will take place, i.e. comparison of mean VAS scores between sleep conditions, as well as mean EEG activity between sleep conditions. Second, the dynamics of the data will be analyzed, taking into account that VAS and EEG vary with time. This will be done with an exploratory data analysis approach. The purpose of looking into the dynamics of the VAS scores and EEG activity is to uncover potential associations between pain and sleep that are not visible in the static analysis. The combination of the two approaches will give a more nuanced view of the potential ways in which pain perception is linked to changes in EEG activity.

The following hypotheses were made for the static analyses:

1) The static VAS scores will *increase* after restricted sleep, as compared to habitual sleep.
2) The change in perceived pain intensity will be accompanied by changes in the static EEG indices. Specifically, there is an expectation to find:
    a. an *increase* in theta activity after restricted sleep as compared to habitual sleep.
    b. a *decrease* in alpha activity after restricted sleep as compared to habitual sleep.
    c. an *increase* in beta activity after restricted sleep as compared to habitual sleep.
    d. a change in delta and gamma activity after restricted sleep as compared to habitual sleep.

The directions of the predicted changes are based on the presented literature. The hypotheses regarding the delta and gamma bands are directionless, as there is insufficient support for a specific direction.

The following hypotheses were made for the exploratory dynamic analyses:

3) The dynamic VAS scores will show a *higher rate of increase* over the 2-min experimental pain stimulation after restricted sleep as compared to habitual sleep.
4) Changes in the dynamic pain scores will be accompanied by changes in the dynamic EEG indices.

Method

Data collection was conducted between March (2013) and October (2014) by STAMI (National Institute of Occupational Health, Oslo, Norway). The research project was approved by the Norwegian Regional Committee for Medical Research Ethics (Region South-East B, approval # 2012/199). The subjects participated in the following tests in a standardized order; electrical pinprick pain, contact heat pain, cold pain, pressure pain, and pain inhibition. This paper will focus on the contact heat pain stimulus protocol. Further information regarding the complete experimental design can be found in Matre et al. (2017). A discussion regarding the strengths and weaknesses of the methods employed in this thesis can be found in the method discussion.

Study subjects

The aim of the research project at STAMI is to investigate the health effects of shift work. Therefore, the subjects of this study are shift workers. Various occupations of shift workers were considered but could not be included due to practical difficulties, e.g. the travel distance between their workplaces and the STAMI research lab. The research group chose to recruit a cohort of nurses.

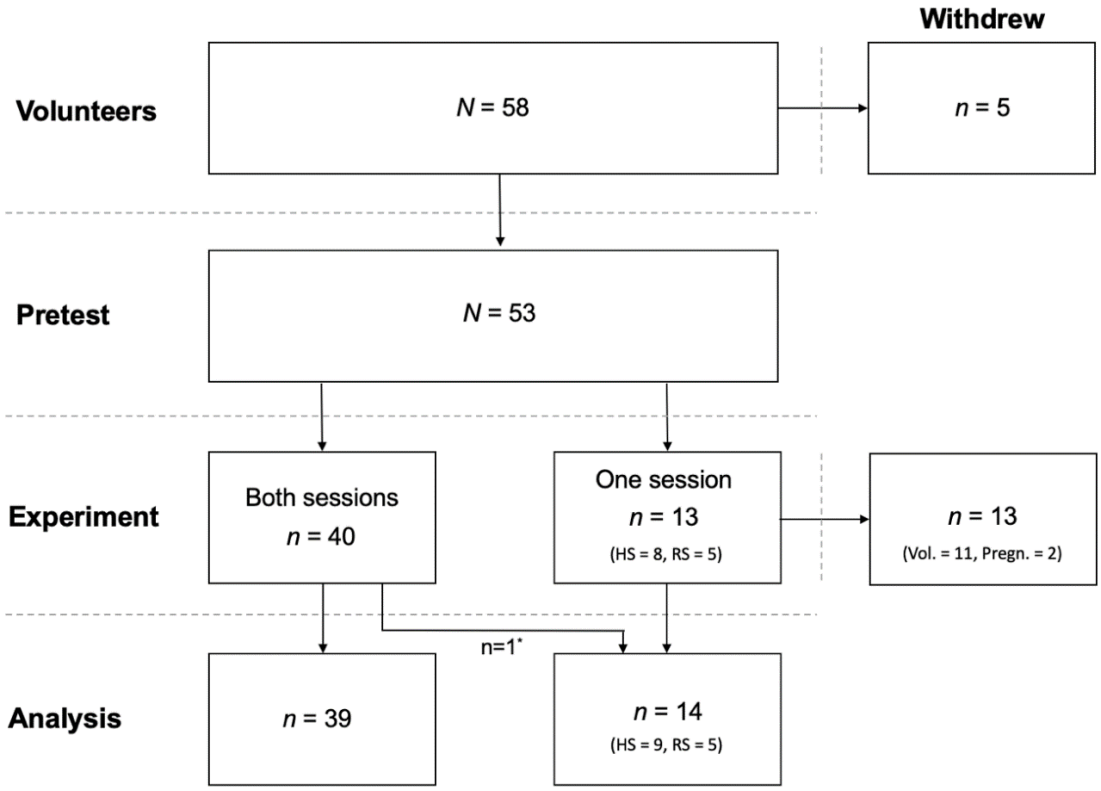
The nurses were recruited from major hospitals in the Oslo area, through wall postings or brief bulletins at the hospitals' respective intranet pages. The exclusion criteria for participation in the study was as following; (1) Pain with intensity ≥3/10 lasting ≥3 months during the last two years, (2) having psychiatric, neurologic, heart or lung disease (well-regulated asthma allowed), (3) headache of moderate intensity for >2 days per month on average, (4) regular use of over-the-counter analgesics, (5) hypertension (>140/90 mmHg)

and (6) being pregnant or breast feeding (Appendix A). All subjects signed a written informed consent form and received economical compensation for their participation in the study (Appendix B).

In total, 58 self-reported healthy nurses volunteered to take part in the experiment. Five of the volunteers withdrew before the first experimental session, leaving 53 subjects who participated in the first experimental session (*age range* 24-57 years, *mean* = 31.6, *SD* = 9.0, 41 women). Out of these 53 subjects, 40 participated in both sleep conditions and completed the study. Five subjects dropped out after the restricted sleep condition (RS), and eight subjects dropped out after the habitual sleep condition (HS) (Figure 4).

Figure 4

Flowchart of study participants



Note. Five subjects withdrew before the pretest. Eleven subjects withdrew due to personal reasons after participating in the first sleep condition, two subjects had become pregnant and did no longer meet the inclusion criteria. *One participant was moved from the subsample "both sessions" to "one session" due to missing pain score data from the restricted sleep session. HS = Habitual sleep, RS = Restricted sleep.

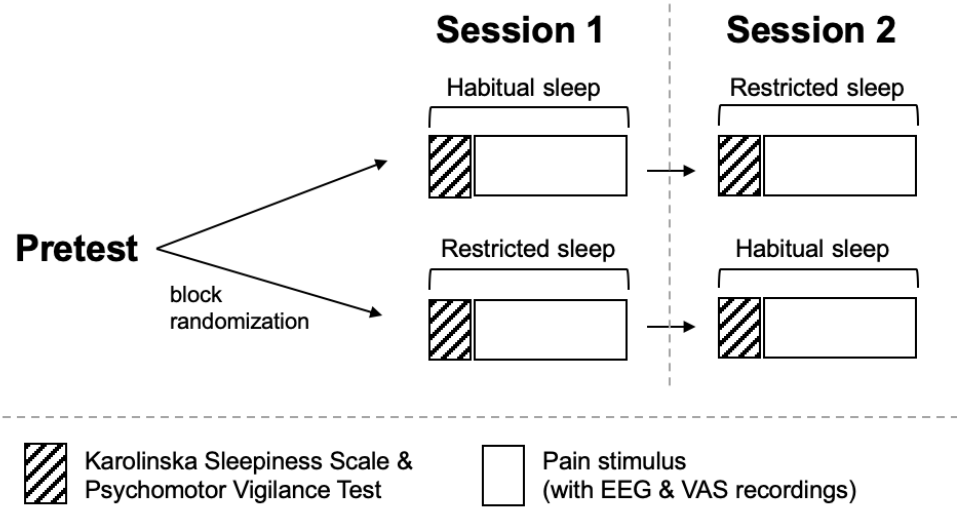
Prior to analysis, one participant was moved from the subsample "participated in both sessions" to "participated in one session" due to missing pain score data from the restricted sleep session. In the statistical analysis, a total of 39 subjects were included in both sleep conditions and 14 subjects were included in only one sleep condition (n=9 for HS, n=5 for RS). The habitual sleep condition had a total of 47 unique participants (*age range* 24-57 years, *mean*=31.98, 33 women), while there were 45 unique participants in the restricted sleep condition (*age range* 24-57 years, *mean* = 31.98, 36 women).

Experimental protocol

The experimental design was a paired cross-over design with block randomization (Figure 5). Two days prior to the first experimental session, a pre-test session was conducted to familiarize subjects with the experimental protocol. Each subject participated in two experimental sessions. One session was preceded by a restricted sleep condition, while the other session was preceded by a habitual sleep condition. In all experimental sessions, EEG data and pain scores were recorded while subjects underwent a contact heat pain stimulation protocol lasting two minutes. All experimental sessions were carried out by the same experimenter. The experimenter was kept blinded with regards to sleep conditions.

Figure 5

A schematic overview of the experimental process



Note. The study has a within-subject cross-over design with counter balanced order of sleep conditions. Subjects participated in an initial pretest and two experimental sessions, where they received the same experimental protocol after two nights of habitual sleep and after two nights at work.

The two sleep conditions were carried out as follows: Restricted sleep was defined as at least two consecutive nights at work, and the habitual sleep condition was defined as at least two nights of habitual sleep. Most all subjects had ≥ four nights of habitual sleep before the habitual sleep condition session, except for three subjects who had their last night shift three days before the experiment. As for the restricted sleep condition, most subjects worked two consecutive nights (n = 29), some worked three consecutive nights (n = 13), and a few worked four consecutive nights (n = 3) before the experiment. All subjects came directly from work to STAMI after undergoing restricted sleep (≤60 minutes with public transport). All subjects were instructed to abstain from alcohol in the 24-hour period prior to both experimental sessions.

Sleepiness

At the beginning of each experimental session, sleepiness was measured using the Karolinska sleepiness scale (KSS) and the psychomotor vigilance test (PVT). Results from KSS and PVT were used to evaluate the effects of the restricted sleep condition.

KSS is a one-dimensional scale, with end points 1 = “extremely alert”, and 9 = ”very sleepy, fighting against sleep” (Åkerstedt & Gillberg, 1990). Subjects indicate their answers by saying an integer between 1 and 9.

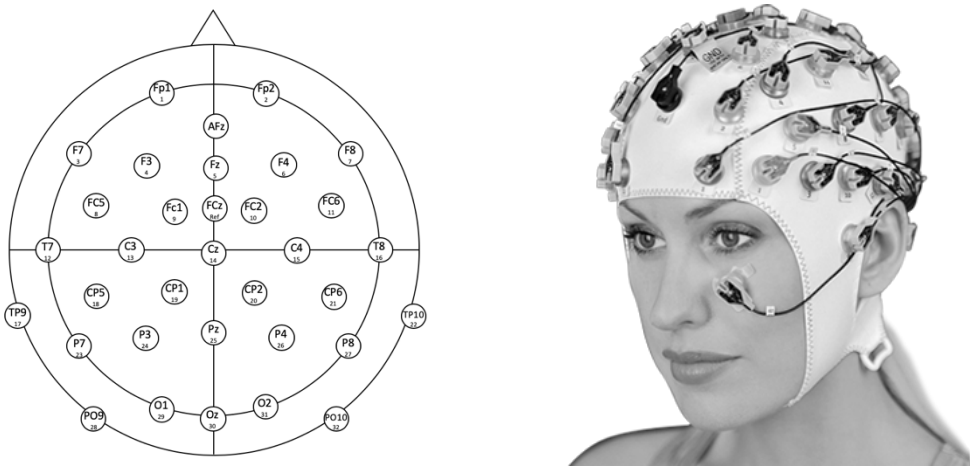
PVT is a behavioral alertness measure using a computerized version of the 10-minute psychomotor vigilance test. PVT is considered an objective measure of sleepiness, and consists of repeated measures of selected parameters of cognitive factors that are sensitive to sleep loss (Basner & Dinges, 2011). Prior to the PVT, subjects were instructed to focus on the computer screen and press the response button as soon as a white colored numbered appeared in a rectangular box on the red screen. Intervals between each lap varied form 2-10 seconds after button-press. The following measures were calculated; mean reaction time (RT), mean 10% fastest RT, mean 10% slowest RT, and the associated inverse measure Mean 1/RT. The inverse reaction time was used as the preferred parameter of sleepiness (Basner & Dinges, 2011).

EEG recordings

EEG was recorded by a standard soft 32-channel cap with active electrodes (actiCAP, Brain Products GmbH, Gliching, Germany) placed according to the international 10-20 system matching the subjects head size. The continuous EEG signal was recorded at 2 kHz and digitally amplified (QuickAmp 40-channel amplifier and Brain Vision Recorder, Brain

Products GmbH, Gliching, Germany). The common reference electrode was placed at FCz, and two surface electrodes were placed at the upper left (VEOG) and lower right (HEOG) side of the eye in order to monitor ocular movements and eye blinks. Impedance was kept below 5 kΩ. The experimenter had a written protocol, ensuring that all subjects were given the same information and instructions. Subjects were seated and had their eyes open during the experimental protocol.

Figure 6



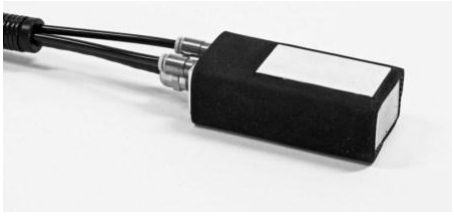
Note. Left: Placement of 32 electrodes according to the international 10-20 system. Right: A woman wearing an electrode cap. One electrode is tracking the movements of her left eye. Images courtesy of Brain Products GmbH, Gliching, Germany.

Heat stimulation and pain6

Contact heat pain was delivered by a 12.5 cm^2 peltier thermode (MSA-II, Somedic AB, Solna, Sweden). The thermode was attached to the volar forearm with a blood pressure cuff inflated to 20 mmHg.

Figure 7

The thermode used in the experiment



Note. The thermode has a 25 x 50 mm (12,5 cm^2) active area.

In an effort to standardize the experienced pain intensity in the experiment, the temperature of the heat stimulation was individualized. This was done by determining each subject's "pain6" temperature during the pretest session. The pain6 temperature from the pretest session was used in the subsequent experimental sessions.

Pain6 was defined as the temperature which induced a pain intensity of 6 on a 0-10 verbal numeric rating scale (NRS, endpoints 0="no pain", 10="worst imaginable pain"). To determine pain6, the subjects first received three 7 second heat stimuli at 45°C, 46°C and 47°C. If either of these temperatures induced a pain intensity of 6, this temperature was set as their pain6. If pain ratings were below 6 at these three temperatures, subjects received heat stimuli of 48°C and 49°C. Additionally, if the initial temperatures induced a pain intensity above 6, the subjects received heat stimuli of 44°C and 43°C. The outer limits of the heat stimuli were set to 43°C and 49°C.

In the experimental sessions, the temperature of the thermode begun at 32°C and increased with 1°C/sec increments until it reached the subject's pain6 temperature. The temperature then stayed at the pain6 temperature for 120 seconds before cooling off. All study subjects continuously rated the intensity of the pain during the period of heat stimulation with a joystick lever connected to a 10 cm visual analogue scale (VAS). The scale endpoints were defined as "no pain" and "worst imaginable pain". Pain ratings were sampled electronically with a sample frequency of 1 Hz. Pain scores are given in cm and will be referred to as VAS scores.

Data analysis

The EEG signal was re-referenced to linked mastoids (electrodes TP9 and TP10), downsampled to 512 Hz and filtered (0.5-100 Hz) using BrainVision Analyzer (BrainVision Analyzer, Brain Products GmbH, Gliching, Germany). Following this, the EEG files were exported for further pre-processing in MATLAB (MATLAB R2019a, The MathWorks, Inc., Natick, Massachusetts, United States) and EEGLAB (EEGLAB version 2019.1.0, available at sccn.usd.edu/eeglab), an open source toolbox running under the MATLAB environment (Delorme & Makeig, 2004).

In addition to the files containing EEG data, time stamps indicating events like turning on/off test equipment and stimulus onset were imported to MATLAB. In order to signify the beginning and end of the time period to be analyzed, the time stamps were used to add markers in each subject's EEG file. The time period to be analyzed was defined by the two-

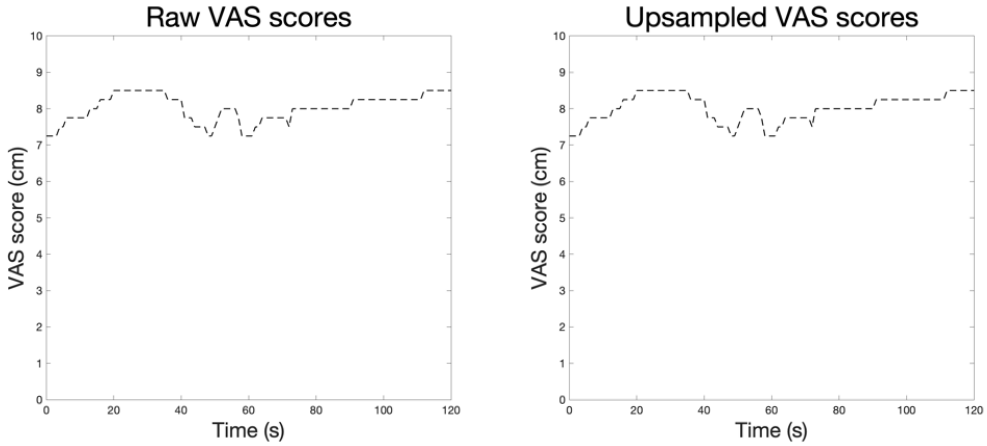
minute period of pain6 heat stimulation. See Appendix C and D for MATLAB scripts made to import and add time markers.

Importing VAS scores

VAS-scores were imported to MATLAB and upsampled to 512 Hz to match the EEG-sampling rate. A random selection of the upsampled VAS-scores were visually compared with the original VAS data to validate this procedure (Figure 8).

Figure 8

Comparison of original VAS scores sampled at 1 Hz and upsampled VAS scores at 512 Hz



Note. Left panel: Original VAS scores from a randomly selected subject. Right panel: Upsampled VAS scores for the same subject.

The VAS scores for each subject were then added to the subject's EEG file in the form of an additional channel, making 33 channels in total. Adding the VAS scores as a separate channel made sure of accurate synchronization between the VAS-scores and the EEG-signal. See Appendix C and D for MATLAB scripts made to import and add VAS scores.

To further ensure that VAS scores were imported correctly, the mean of the VAS scores based on the whole two-minute period was calculated for each subject. These, as well as the VAS scores from the first and last second of the two-minute time period, were subsequently investigated. With a heat-stimulus temperature of pain6, the rated pain intensity should be above zero throughout the whole two-minute period. All extracted VAS scores should consequently be above zero. One subject was found to have a VAS score of zero throughout the whole two-minute period. The source file of this subject's VAS scores and the notes from the experimental session were checked, and an error was found in the time markers. The error was caused by turning the EEG equipment on, off, and on again during the experiment due to

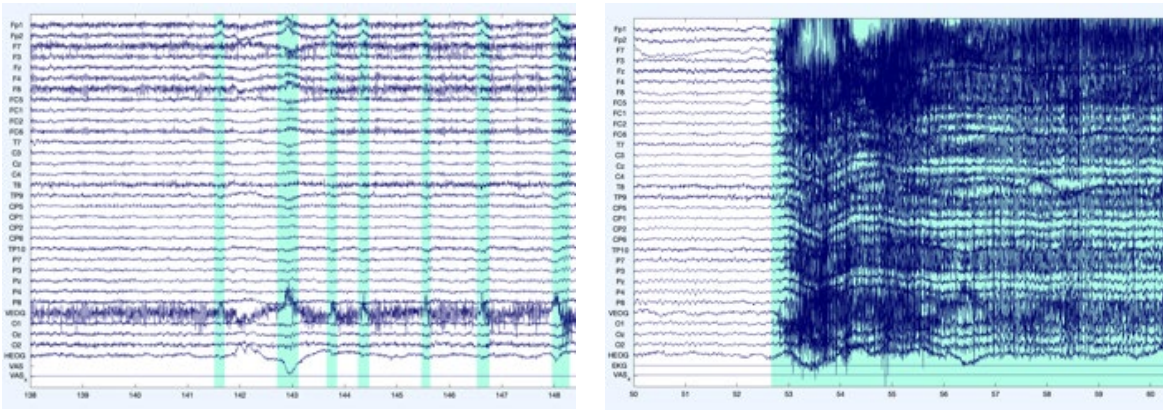
technical issues. The time markers were corrected manually to correspond with the events in the experiment.

Artifact removal and filtering

Following this, all EEG files were subjected to artifact removal. Artifacts are defined as signals recorded by EEG, but which are not generated by the brain. Artifacts include activity occurring as a result of eye movements and eye blinking, EKG pulse, as well as other factors such as myogenic activity and speech. As the EEG data in question was continuous, the issue of losing data with artifact rejection was not of a primary concern. Other automatic methods of artifact removal, like ICA (independent component analysis) were considered, but found to be unsuitable for continuous EEG data which was not divided into epochs (*Chapter 01: Rejecting Artifacts*, 2019). "Continuous removal by eye" was thus chosen as the preferred method of artifact rejection. Artifacts were removed using the EEGLAB interface for continuous artifact removal by eye. Artifacts caused by eye blinking, as well as stretches of time characterized by noise across many channels, were marked and rejected (Figure 9). As the artifact removal was done by rejecting parts of the signal, signal length will vary between subjects and sessions. Prior to analysis, a threshold value of minimum 20 seconds signal length was set as an inclusion criterion for the statistical analysis. The method of continuous artifact removal by eye was employed with training and supervision by supervisor Petter Moe Omland (M.D).

Figure 9

An example of artifact removal



Note. The bright green color represents the areas marked for rejection. Left: Artifact removal of multiple eye blinks. Muscle artefacts can still be seen in the frontal channels. Right: Removal of a larger area of the signal characterized by artifacts generated by movement or speech etc.

The EEG data was further processed by filtering in order to remove line noise occurring around 50 Hz. The standard notch filter provided by EEGLAB is known to create distortions around the filtered spectrums (Mitra & Bokil, 2009), and other methods are therefore recommended. The filtering was thus done with the MATLAB compatible "CleanLine" function, developed by Tim Mullen (available at https://www.nitrc.org/projects/cleanline).

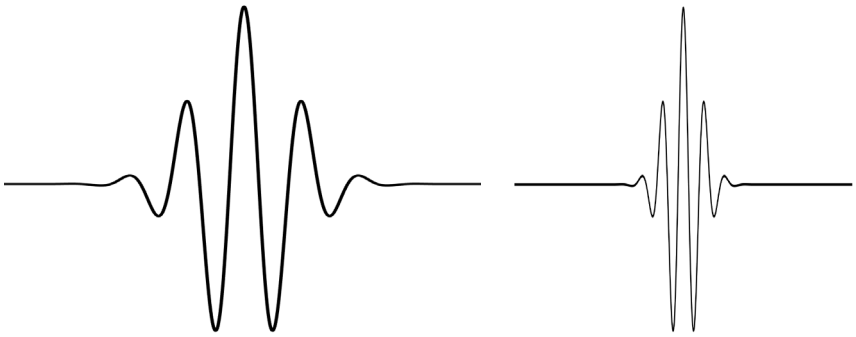
Spectral analysis

Spectral analysis is a method used to analyze EEG signals by quantifying the amount of oscillatory activity of different frequencies in the EEG signal (Do-Won & Chang-Hwan, 2018). To retain information about spectral dynamic over time, a continuous wavelet transform was carried out in MATLAB. The wavelet-function was provided by C. Torrence and G. Compo (available at http://atoc.colorado.edu/research/wavelets/). In short, continuous wavelet transform (CWT) can be used to analyze EEG data by extracting mean power differences from the different frequency bands. This is done by sequentially comparing small segments of the EEG signal with a chosen wavelet and calculating the similarity between the signal and the wavelet. A wavelet is a mathematical function that can be used to analyze continuous time signals.

Wavelet analysis is suggested to be a better approach to spectral analysis than the Fourier transform (Akin, 2002). One of the advantages of wavelet-based analysis is its ability to estimate the power of transient signals without a loss of frequency resolution (Bassani & Nievola, 2008). Estimation of spectral power in CWT is reliant on the chosen mother wavelet and its scaling and shifting properties. The Morlet wavelet was chosen as the mother wavelet in this analysis (Figure 10), as it is a complex wavelet function and thus better adapted for capturing oscillatory behavior, such as brain activity (Torrence & Compo, 1998).

Figure 10

The Morlet wavelet at two different scales



The wavelet mimics different frequencies by scaling up and down, and the range of the scales can be set to match the frequency range that is present in the signal. The comparison between the wavelet and the signal is run multiple times, each time at a different scale.

The continuous wavelet transform was applied to each channel of the EEG data in both sleep conditions for all subjects. The spacing between the discrete scales for the transform were adjusted from default settings to obtain a better scale resolution. The smallest scale of the wavelet was set to correspond to approximately 0.5 Hz. Following the transform, scales were translated to their corresponding frequencies (Table 1). The wavelet coefficients were split into the following frequency bands; delta (1-4 Hz), theta (4–8 Hz), alpha (8-12 Hz), beta (12-32 Hz) and gamma (32–70 Hz).

Table 1

Ranges for wavelet scales and their corresponding EEG bands

EEG band	Frequency Range (Hz)	Corresponding Scale Range
Delta (δ)	1-4	[0.783-0.211]
Theta (θ)	4-8	[0.201-0.102]
Alpha, (α)	8-12	[0.097-0.069]
Beta (β)	12-32	[0.066-0.026]
Gamma (γ)	32-70	[0.025-0.012]

CWT results in a matrix of complex numbers. One part of each number represents the similarity of the signal (at a given time) to the Morlet wavelet (at a given scale). The other part represents the phase of the signal (at the given time). The sign of the value gives additional information regarding the phase. For further analysis, the absolute values of the wavelet coefficients were used. For each EEG channel, all the wavelet coefficients that belonged within the same frequency band were summed together. Following this, all coefficients within the same frequency band were summed across channels, in order to reduce the amount of data for further statistical analysis. With 92 unique sessions (39 subjects with two sessions and 14 with one session) and 5 frequency bands, this resulted in a total of 460 observations. These observations represent the static EEG indices. See Appendix E for the MATLAB script used to run CWT and prepare the static data.

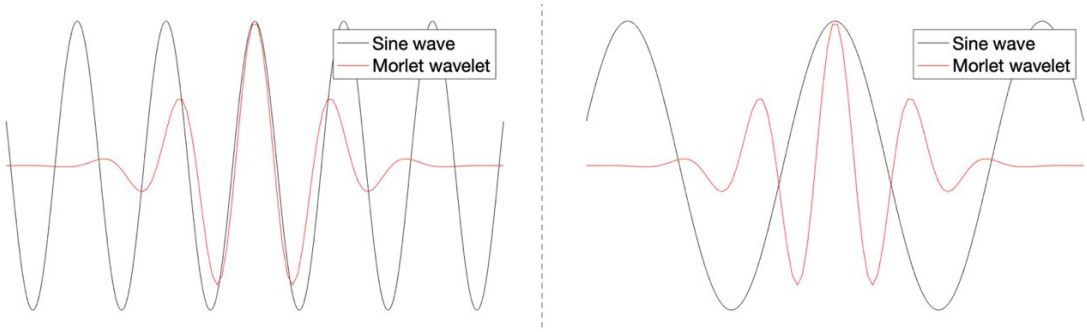
To look into the dynamics of the EEG signals, the two-minute period of pain stimuli was divided into partially overlapping 15 second time periods. The overlap between two consecutive 15 second windows was set to 50% (Gram et al., 2015). This time series was then subjected to the continuous wavelet transform. Wavelet coefficients for each 15 sec time

series were collated in a 4D-matrix and summed within and across channels for each frequency band. These observations represent the dynamic EEG indices. Mean VAS-scores were calculated for the corresponding 15 sec intervals. These observations represent the dynamic VAS scores. See Appendix F for the MATLAB script used to run CWT and prepare the dynamic data.

With CWT, the activity within the EEG bands is measured in terms of similarity between the Morlet wavelet and the EEG signal (Figure 11). The CWT coefficients are obtained by computing the product of the signal with the shifted and scaled Morlet wavelet and then integrating the result. When the wavelet is at a scale that closely resembles the oscillations that are present in the EEG signal, the absolute value of the CWT coefficient is large (phase shifting affects the sign of the value). When the wavelet is at a much larger or smaller scale than the oscillations found in the EEG signal, the value of the CWT coefficient is near zero. Although the CWT coefficients are a measure of similarity, they cannot be interpreted directly as correlation coefficients.

Figure 11

Two Morlet wavelets (of the same scale) compared to two sine waves (of different frequencies)



Note. The sine waves represent the oscillatory brain activity. The two comparisons shown in this figure would lead to different CWT coefficients. The comparison between the sine wave and the Morlet wavelet in the left panel would result in a CWT coefficient with a higher absolute value than the comparison in the right panel. The is due to the fact that the sine wave in the left panel more closely resembles the given scale of the Morlet wavelet.

The experimental design does not allow for observations regarding the general level of EEG activity in the frequency bands, as there is no baseline condition to compare with. It is, however, possible to tell if there has been an increase or decrease in activity after restricted sleep as compared to after habitual sleep within each of the frequency bands.

When presenting and discussing the results in this thesis, I will refer to static and dynamic EEG indices. "Static EEG indices" represent the CWT coefficients within each of the frequency bands, summed over the 2-min period. "Dynamic EEG indices" represent the CWT coefficients within each of the frequency bands, summed over the 15 second intervals. Similarly, "alpha activity" means the power of the CWT coefficients of the alpha frequency band. The higher the power of the CWT coefficients in a particular frequency band; the more oscillatory activity of that frequency range has been detected in the EEG signal. Thus, an increase in the power of the CWT coefficients represents an increase in EEG activity. Absolute activity and changes in absolute activity will be reported in arbitrary units (au). "A one arbitrary unit increase in alpha activity" corresponds to an increase of 1 in the value of the summed CWT coefficients of the alpha frequency band.

Statistics

Statistical analyses were performed in Stata 16 (StataCorp, College Station, TX, USA). The author of the thesis was kept blind to which subject files belonged to which sleep condition during the entirety of the pre-processing and statistical analyses. For all analyses, *p*-values < 0.05 were considered significant. P-values and confidence intervals are reported where applicable.

Linear mixed model analysis of VAS scores and static EEG indices

A mixed model analysis was used for paired comparisons of the static VAS scores between the habitual sleep condition and the restricted sleep condition, as well as for the static EEG indices. In addition, a mixed model was used for paired comparison of the dynamic VAS scores between the habitual sleep condition and the restricted sleep condition. For the dynamic VAS analysis, VAS was set to be the dependent variable, and sleep condition and time were included as independent variables, as well as an interaction term. Time was included in order to check if the VAS scores followed a significantly different pattern in one of the sleep conditions, i.e. whether the increase in pain intensity was steeper.

Linear mixed models were chosen over a t-test in order to include subjects who only participated in one of the two experimental sessions. A regular t-test follows the complete case principle (CC) and would exclude these subjects due to missing data points. The approach taken in this thesis is the intention to treat (ITT) principle, which does not exclude subjects who fail to complete the experiment. Rather, these are included in the analysis in

order to avoid various biases that can arise when excluding subjects (Hollis & Campbell, 1999).

A mixed model analysis handles missing data by making inferences under the "missing at random" (MAR) assumption. The MAR assumption is considered true for this dataset, as the withdrawn subjects did not cause a substantial change of the descriptive statistics (age mean, age range, gender) of the data in the two sleep conditions. However, as there is no direct way to test the MAR assumption (Potthoff et al., 2006), one should be cautious of generalizing inferences from the results to the general population.

A few other assumptions should be met in order to apply a linear mixed model to a dataset. The dependency structure of the model must be modelled correctly, and secondly, random effects and within unit residual errors should follow normal distributions and have a constant variance. While some of these assumptions can be checked, linear mixed models hold up quite robustly to violations of the second assumption (Verbeke, 1997).

The dependency structure in this dataset is as follows: Measurements pertaining to the same individual are considered dependent on each other, while data gathered from different individuals are considered independent of one another. It could be argued that other dependencies exist, such as clusters of individuals from the same place of work. However, the focus of this thesis is to investigate the effect of restricted sleep on pain perception. Other contextual factors of a of psychosocial nature, such as place of work or type of shift rotation should be considered for future studies.

The residual errors of the models were checked with Q-Q plots and found satisfactory (Appendix G).

Visual inspection and linear mixed model analysis of dynamic EEG indices

The dynamic EEG indices were investigated with the use of an explorative data analysis approach (EDA). EDA states that it is central to get a sense of the data by visualizing it before subjecting it to further statistical testing (Evans, 2007). Visualizing the data set is an important tool for gaining valuable insights into the data and can lead you to uncover underlying structures and detect important variables (Evans, 2007). Furthermore, the initial exploration of the visual data can aid you in choosing the appropriate statistical tests.

In the first phase of the dynamic analyses, mean activity within each frequency band, based on all study subjects, was calculated and plotted along the time dimension. Each plot was visually inspected with regards to 1) patterns of increase and/or decrease in activity, and 2) the adjoining VAS score dynamics. Similar plots were made for the four participants with

the largest VAS_{delta}, i.e. the most prominent difference in VAS score between the beginning and the end of the two-minute period of pain6 stimuli. The four VAS_{delta} subjects were investigated in order to see if a lager increase in VAS scores throughout the experimental session would result in a more evident relationship between the perceived pain intensity and the activity within each frequency band. VAS_{delta} was calculated by subtracting the mean VAS score of the second 15 sec interval (t_2) from the mean pain score of the last 15 sec interval(t_{end}). t_2 was chosen over t_1 because the thermode reaches the pain6 temperature just before t_1. The VAS scores in the t_1-interval is influenced by this recent incline in temperature to a varying degree amongst the subjects. Hence, t_2 gives a more accurate picture of the pain intensity at pain6 temperature.

Inspecting the data plots depicting dynamic EEG activity yielded no strong leads that could explain the relationship between VAS scores and sleep restriction. Following the visual inspection, each frequency band was tested in a separate mixed model. Each model included VAS scores as the dependent variable. Sleep condition and one of the five frequency bands were included as independent variables. The interaction effect between sleep condition and EEG activity was also included. Time was included as a control variable.

Results

Artifact removal

Descriptive statistics showed that all subjects had a signal length of more than 20 seconds and could be included in the analysis (Table 2).

Table 2

Descriptive statistics of EEG signal length (sec) post artifact removal

Sleep condition	Mean signal length (s)	SD (s)	Range (s)
Habitual sleep	81.29	13.49	[54.48, 105.82]
Restricted sleep	73.63	16.33	[27.11, 104.64]

Sleepiness

Sleepiness was increased with 2.9 points after restricted sleep, as measured on the 1-9 Karolinska sleepiness scale (Table 3). Reaction time had increased by 0.03 seconds following restricted sleep relative to habitual sleep (Table 3). The results of these measures indicate that

the subjects were significantly more sleepy in the restricted sleep condition than in the habitual sleep condition.

Table 3

S*ummary of Wilcoxon signed-rank tests and descriptive statistics of subjective sleepiness and inverted reaction time*

	Habitual sleep (HS)		Restricted sleep (RS)		z[a]	P-value[a]
	Mean	SD	Mean	SD		
Subjective sleepiness (KSS)	3.92	1.67	6.82	1.06	-5.2	< 0.001
Reaction time (RT)	0.39	0.07	0.42	0.09	3.2	0.001

Note. The descriptive statistics include the full sample ($N = 53$). [a] Wilcoxon signed-rank tests are calculated based on the subjects who participated in both experimental sessions ($n = 39$).

Analysis of static VAS scores

The linear mixed model analysis estimated an increase of 1.05 cm (95% CIs [0.49, 1.60], $p < .001$) in mean VAS score after restricted sleep, relative to habitual sleep. This corresponds to an estimated 24.9% increase in pain intensity. Descriptive statistics are shown in Table 4.

Table 4

Descriptive statistics of VAS-scores

	Habitual sleep (HS)		Restricted sleep (RS)		Mean Difference	Percentage change
	Mean	SD	Mean	SD		
VAS	4.67	2.51	5.88	2.55	1.21	25.90

Analysis of static EEG indices

None of the five frequency bands showed a significant mean change in absolute activity relative to sleep condition. Results from the linear mixed models are summarized in Table 5. Positive coefficients indicate a higher level of absolute activity in the restricted sleep conditions as compared to the habitual sleep condition. Descriptive statistics of absolute activity within the two sleep conditions and estimated changes in absolute activity is visualized in Figure 12. Model summaries for each of the five mixed models can be found in appendix H.

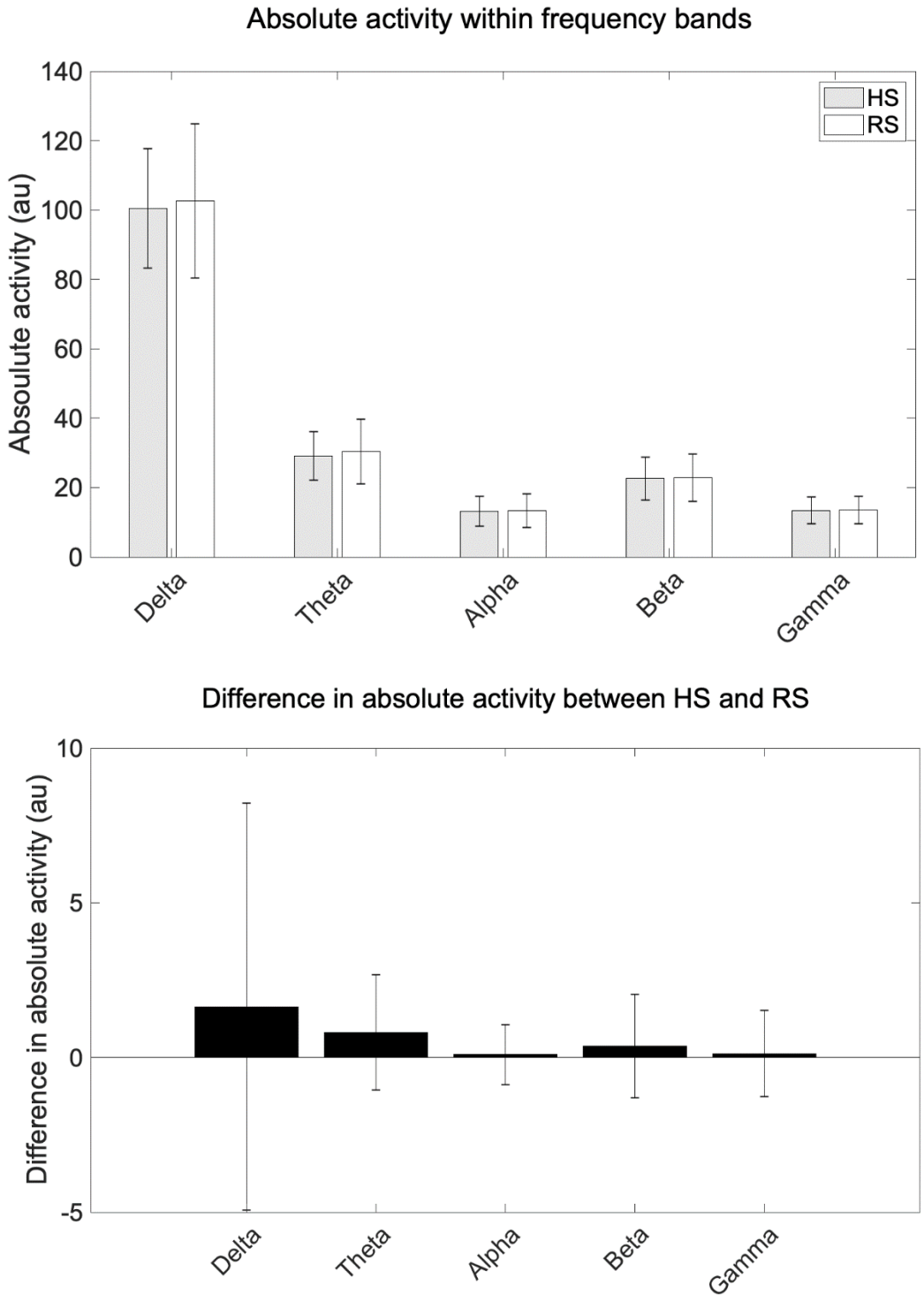
Table 5

Linear mixed model summary of how sleep condition is associated with changes in activity within each of the five frequency bands

Dependent variable	Coefficient [a + b] (absolute activity in au)	95% CI [a]	*p*-value
Delta	1.64	[-4.94, 8.22]	0.626
Theta	0.81	[-1.05, 2.67]	0.394
Alpha	0.09	[-0.87, 1.06]	0.850
Beta	0.36	[-1.31, 2.03]	0.671
Gamma	0.13	[-1.26, 1.52]	0.858

Note. [a] Coefficients and CIs in this table are divided by e+07. [b] Coefficients represent the mean change in absolute activity (in au) within a frequency band between sleep conditions. HS = Habitual sleep, RS = Restricted sleep. The reference group for the sleep condition is habitual sleep. Positive coefficients indicate a higher level of absolute activity in RS than in HS. All frequency bands were tested in separate models. Each model included one of the five frequency bans as the dependent variable and sleep condition as the independent variable. Model summaries for each of the five mixed models can be found in appendix H.

Figure 12



Note. Top: Average absolute EEG activity (with SD) within each frequency band relative to sleep condition. Bottom: Estimated difference in absolute activity (with 95% CIs) within each frequency band between RS and HS. A bar area above the x-axis indicates that the level of activity is higher in RS than in HS. HS = Habitual sleep, RS = Restricted sleep. All values in this figure are divided by e+07.

Analysis of dynamic VAS scores

The VAS scores were estimated to be 1.25 cm higher (95% CIs [0.88, 1.61], $p < .001$) in the restricted sleep condition than in the habitual sleep condition. In addition, time was shown to be a significant predictor of VAS scores, with a 0.08 cm increase in VAS scores per one unit increase in time in the habitual sleep condition ($p < .001$). Furthermore, the mixed model estimates indicate a trend where the rate of increase in perceived pain intensity is dependent on sleep conditions, though this was not a significant finding at $p = 0.109$ (Table 6). This trend is visualized in Figure 13.

Table 6

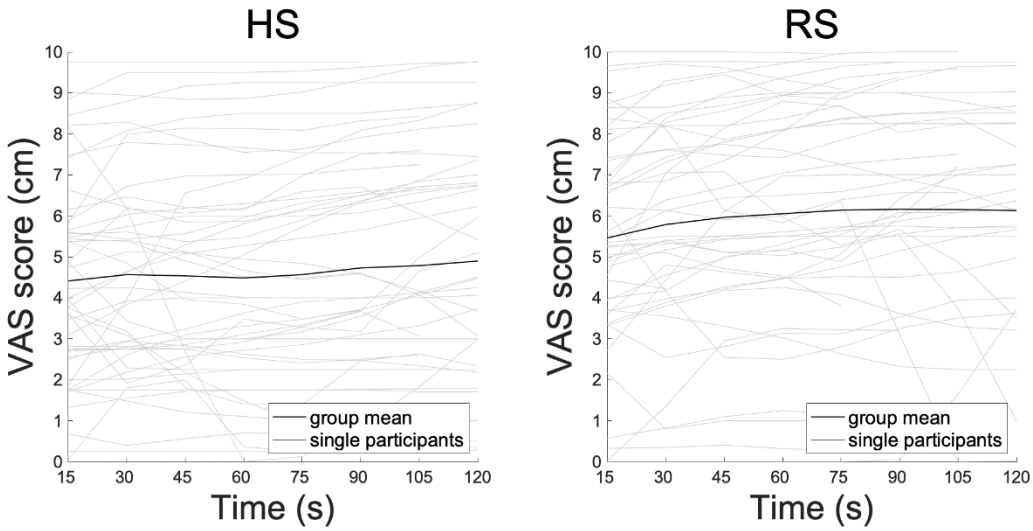
Linear mixed model summary of the effect of sleep and time on dynamic VAS scores

Fixed effects	Coefficient (VAS in cm)	95% CI	*p*-value
Sleep	1.245	[0.879, 1.611]	<.001
Time	0.084	[0.043, 0.125]	<.001
Sleep#Time	-0.041	[-0.103, 0.021]	.109
Cons	4.364	[3.656, 5.068]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. A positive coefficient implies a higher mean value in RS when compared to HS.

Figure 13

Dynamic activity of the VAS scores



Note. The black lines represent the mean based on all subjects within each sleep condition, light grey lines represent single participants. The x-axis represents time (s), the y-axis represents VAS scores (in cm). HS = Habitual sleep, RS = Restricted sleep.

Analysis of dynamic EEG indices

In the static EEG analysis, the EEG activity within each frequency band is reduced to a single mean value. As a result of this, important dynamics in the EEG activity and potential correlations between this activity and the dynamics of the VAS scores are not visible. This necessitates the analysis of dynamic EEG data and VAS scores. The dynamics of the EEG activity and VAS scores are made visible by including a larger number of data points per subject. Using this approach, it is possible to test whether the dynamic EEG indices explain the differences in VAS scores between the restricted sleep condition and the habitual sleep condition.

In this section I will present the graphic representations of data based on all subjects, and data based on the four top VAS_{delta} subjects. Following this I will describe the statistical testing of the dynamic EEG indices.

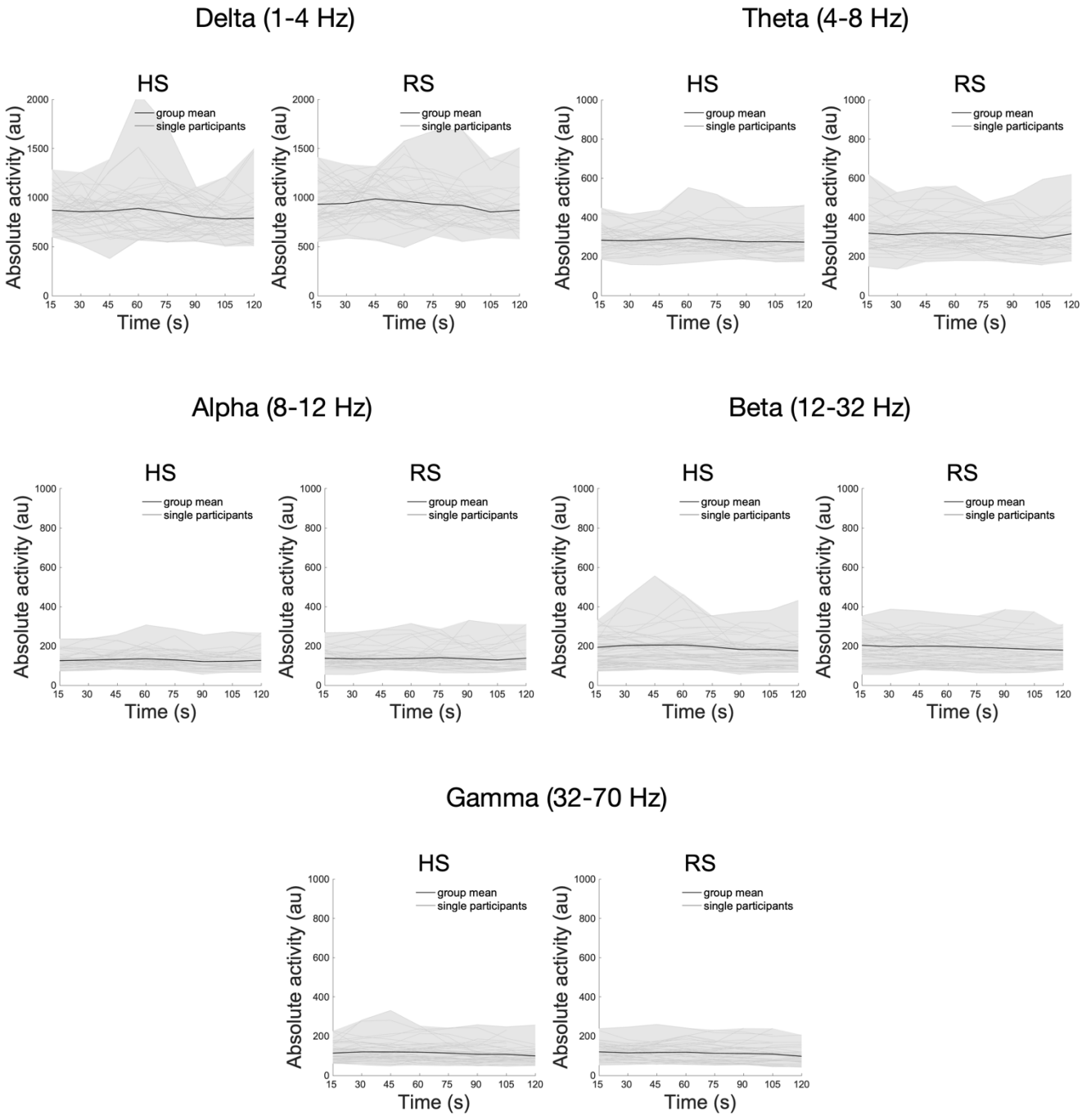
Graphic representation of dynamic EEG indices based on all study subjects

The biggest difference in activity between sleep conditions can be seen in the delta band. The level of activity is higher in the restricted sleep condition than in the habitual sleep condition. However, the mean level of activity in the delta band is overall higher than in the other frequency bands. The range of the standard deviations are also larger (Figure 11, Left panel). This could account for some of the fluctuations.

Overall, the activity in each of the EEG frequency bands showed a high degree of similarity between the two sleep conditions (Figure 14). Furthermore, the mean activity within each frequency band showed no clear patterns that matched the dynamics of the VAS scores seen in Figure 13.

Figure 14

Dynamic EEEG activity based on all subjects



Note. Dynamic activity of the delta, theta, alpha, beta and gamma frequency bands within HS and RS. The black lines represent the mean level of activity based on all subjects within each sleep condition. Light grey lines represent single participants. The light gray area above and below the mean represents the full range of individual activity. The x-axis represents time (s), the y-axis represents absolute activity (in au) within the frequency bands. HS = Habitual sleep, RS = Restricted sleep.

Graphic representation of dynamic EEG indices based on the top four VAS_{delta}-subjects

Activity plots were also made for the four participants with the most prominent difference in VAS scores within a session (VAS_{delta}). Data from two of the subjects were from the habitual sleep condition, data from the other two subjects were from the restricted sleep condition (Figure 15).

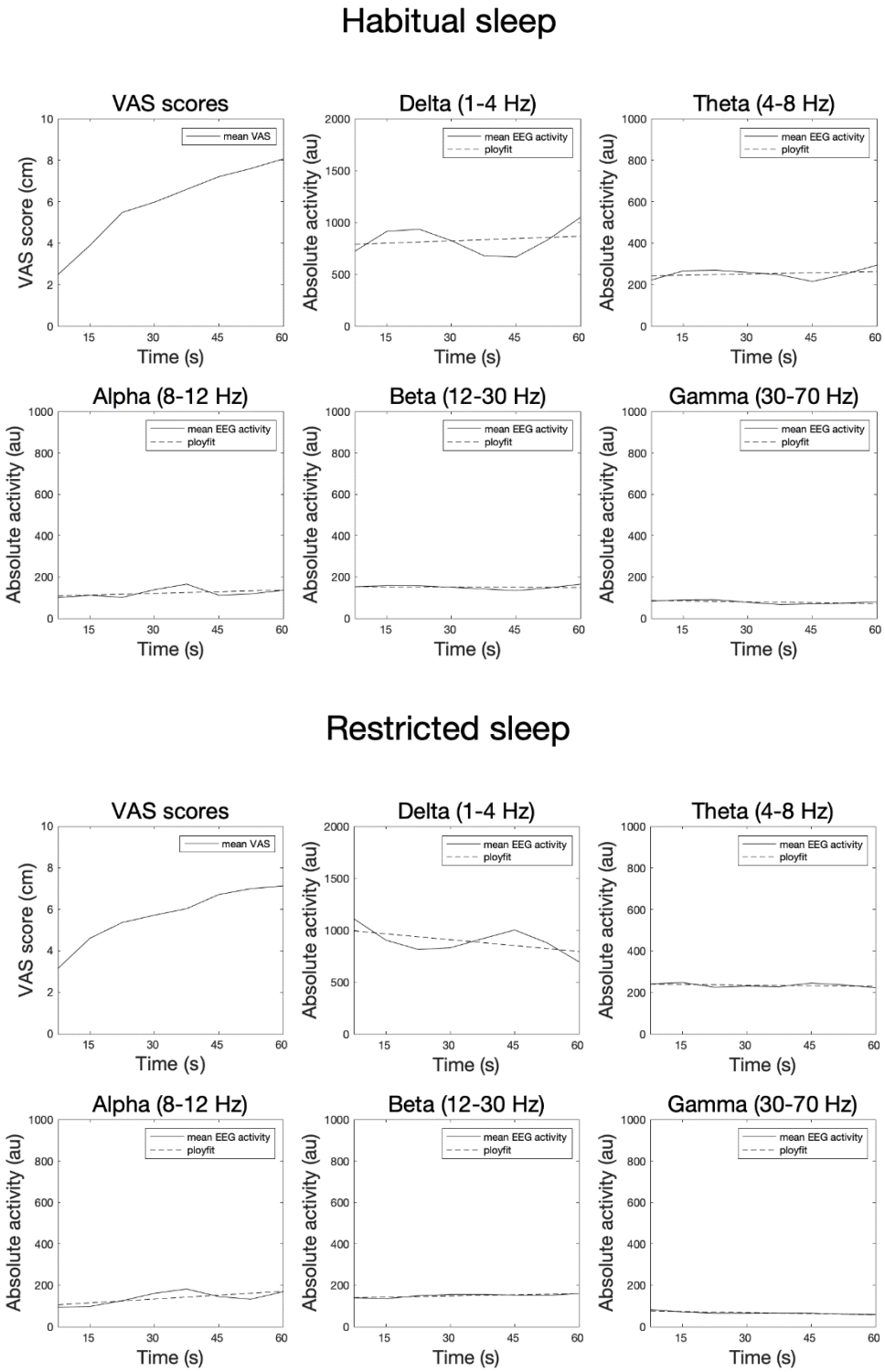
In the habitual sleep condition, the delta band shows a general trend of slight increase in activity over time, but with a dip in activity towards the second half of the two-minute period. In the restricted sleep condition, the delta band has a general trend of decrease in activity from beginning to end. This is a contrast to the trend in the habitual sleep condition. The alpha band shows a general trend of slight increase in activity over time in both of the sleep conditions, and the peak of the alpha activity is found around the middle of the time period. The theta band has a general trend of slight increase in activity over time in the habitual sleep condition, with a dip in activity towards the second half of the two-minute period. In the restricted sleep condition, however, the activity is seen to be quite stable across the two-minute period. The beta band has a relatively stable level of activity throughout in both sleep conditions. The activity in the gamma band is quite stable in the habitual sleep condition but has a dip in activity towards the second half of the two-minute period. The gamma activity is also quite stable in the restricted sleep condition but is leaning more towards an increase in activity than in the habitual sleep condition.

The VAS scores in the habitual sleep condition follow a trend of continuous increase. The rate of increase is larger in the beginning than in the middle and end portion of the two-minute period. The VAS scores also increase steadily throughout in the restricted sleep condition, but with a slightly different curve than in the habitual sleep condition. The continuous increase in pain intensity is not clearly reflected in the visualization of any of the frequency bands.

In short, the VAS scores showed a general continuous increase over time, while the activity of the frequency bands exhibited more variability. None of the frequency bands seemed significantly more promising than others in their ability to explain changes in VAS scores. Neither when based on all subjects, nor when based on the four VAS_{delta} subjects.

Figure 15

Dynamic VAS scores and EEG activity based on VAS_{delta} subjects



Note. The black lines represent mean activity, the dotted black lines represent general trends in the data as calculated by the ployfit function in MATLAB. The x-axis represents time (s) and the y-axis represents absolute activity within the frequency bands (in au). In the plots titled “VAS scores”, the y-axis represents VAS scores (in cm).

The estimated effect of dynamic EEG activity on VAS scores

The goal of the next stage of the analysis was to determine whether the "within subject" aspect of the data would make any potential associations between pain intensity, sleep, and EEG activity visible. This was done by running five separate mixed models, one model for each frequency band. Dynamic VAS scores were set as the dependent variable. Independent variables were sleep condition, EEG activity and sleep x EEG activity. Time was included as a control variable.

The theta frequency band. Theta activity did not explain a significant amount of variance of the VAS scores in the habitual sleep condition (Table 7). However, the interaction term was shown to be statistically significant. The interaction term indicated that the effect of theta activity on the VAS scores was 0.005 cm lower after restricted sleep than after habitual sleep (95% CIs [-0.008, -0.003], $p < .001$). In other words, *decreased* theta activity was associated with heightened VAS scores in the restricted sleep condition (Figure 16, right panel). This effect results in a 0.4 cm increase in VAS when the power of the theta band is increased with 100 au.

Table 7

Linear mixed model summary of variables predicting dynamic VAS-scores (theta band)

Fixed effects	Coefficient (VAS in cm)	95% CI	*p*-value
Sleep	2.571	[1.759, 3.383]	<.001
Time	0.066	[0.034, 0.097]	<.001
Theta	0.001	[-0.002, 0.004]	.557
Sleep#Theta	-0.005	[-0.008, -0.002]	<.001
Cons	6.334	[3.219, 5.270]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. A positive coefficient implies a higher mean value in RS than in HS.

The alpha frequency band. A one arbitrary unit increase in alpha activity was associated with a 0.005 cm (95% CIs [0.001, 0.009], $p = .009$) increase in VAS scores after habitual sleep (Table 8), when controlled for time, i.e. the general increase in VAS that occurs over the two-minute period. The interaction term further indicated that that this effect was 0.006 cm (95% CIs [-0.010, -0.002]) smaller in the restricted sleep condition. This corresponds to a 0.001 cm decrease in VAS scores per one arbitrary unit of increase in alpha activity after

restricted sleep, or a 0.1 cm decrease in VAS scores per 100 arbitrary units of increase in alpha activity. Namely, in the habitual sleep condition, an *increase* in alpha activity is associated with an *increase* in VAS scores, while in the restricted sleep condition, a *decrease* in alpha activity is associated with an *increase* in VAS scores (Figure 16, left panel). The effect that alpha activity has on VAS scores is smaller after restricted sleep than after habitual sleep.

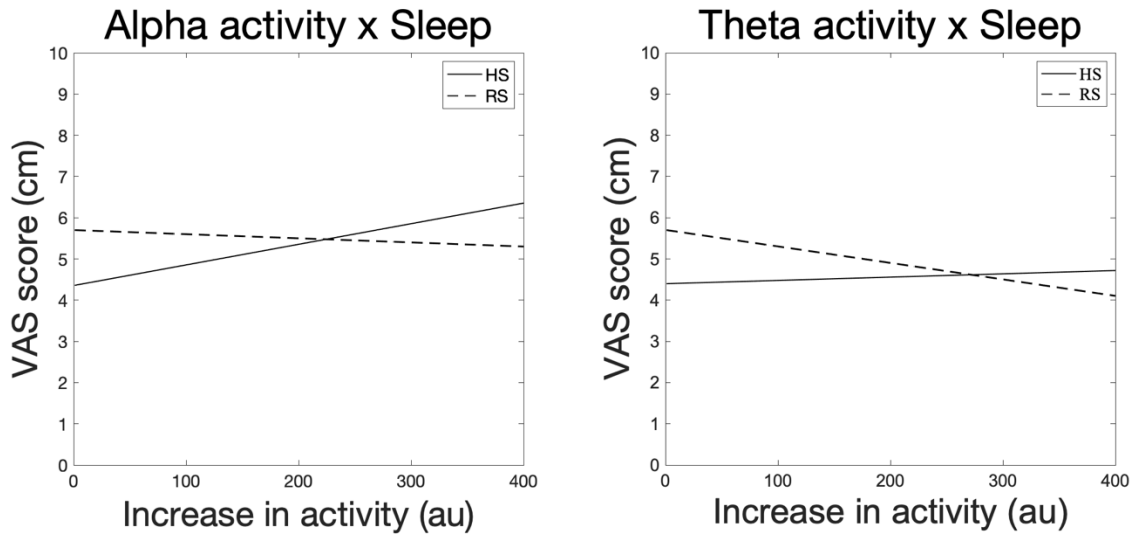
Table 8

Linear mixed model summary of variables predicting dynamic VAS-scores (alpha band)

Fixed effects	Coefficient (VAS in cm)	95% CI	*p*-value
Sleep	1.788	[1.205, 2.372]	<.001
Time	0.068	[0.037, 0.099]	<.001
Alpha	0.005	[0.001, 0.009]	.009
Sleep#Alpha	-0.006	[-0.010, -0.002]	.006
Cons	3.798	[2.953, 4.644]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. A positive coefficient implies a higher mean value in RS than in HS.

Figure 16



Note. Left: The association between alpha activity and VAS scores in HS and RS. Right: The association between theta activity and VAS scores in HS and RS. The x-axis represents increase in activity (in au), the y-axis represents VAS scores (in cm).

The delta, beta and gamma band. The mixed model estimates of the delta, beta and gamma band models showed no significant effect of EEG activity on the VAS scores. Neither did they indicate any significant interaction effects between EEG activity and sleep conditions. Tables containing estimated coefficients, 95CIs and *p*-values for these three respective models can be found in appendix I.

Discussion

The aim of this thesis was to further our understanding of how sleep affects pain perception by studying patterns of activity in the brain. The results confirm previous findings of sleep-induced hyperalgesia, and furthermore, support the notion that alpha and theta activity is associated with the interaction between sleep and pain. The first research objective was to investigate whether perceived pain intensity in response to heat was increased after restricted sleep relative to after habitual sleep. The results of the analyses showed that the perceived pain intensity of a contact heat stimulus was significantly increased after sleep restriction. This effect of sleep restriction-induced hyperalgesia was found both when analyzing static and dynamic pain scores. The second research objective was to identify any patterns in the EEG activity that could be associated with changes in perceived pain intensity. The analyses showed no significant changes in static EEG activity between sleep conditions. However, results showed a significant association between dynamic alpha activity and pain scores. Furthermore, sleep was found to significantly affect the way in which VAS scores were affected by the activity of both the alpha band and the theta band.

Pain intensity

Perceived pain intensity was significantly higher after restricted sleep, compared to habitual sleep (Hypothesis 1 and 3, see “Aims, objectives and hypotheses”). In the static analysis, the pain scores were estimated to increase with about 25% after restricted sleep compared to after habitual sleep. The dynamic VAS analysis was in line with these results. Furthermore, time was indicated to be a predictor of VAS scores, suggesting that pain scores generally increased during the 2-min period of experimental pain, i.e. a trend of temporal summation was seen (Hypothesis 3, see “Aims, objectives and hypotheses”). However, the interaction between sleep and time was not estimated to be a significant predictor of VAS scores. With a *p*-value of .1 this could be due to a low power. This interaction should thus be studied further to confirm whether or not sleep restriction can affect the speed of which the pain intensity increases. In this analysis, time was set as a continuous variable. If one were to

include time as an indicator variable instead, one could gain additional insights into whether there are sleep-dependent changes in *when* the pain increases.

The general finding of sleep restriction-induced hyperalgesia, i.e. increased sensitivity to pain, is consistent with previous findings. A study by Schuh-Hofer et al. (2013) found that one night of total sleep deprivation led to generalized hyperalgesia and a decreased threshold for heat pain. Others have demonstrated how acute sleep deprivation can amplify pain reactivity (Krause et al., 2019). The hyperalgesic effect that sleep restriction has on pain perception is further supported by a number of studies (e.g. Andersen et al., 2018; Finan et al., 2013; Krause et al., 2019).

In the present study, pain intensity was found to increase with about 25% after restricted sleep, compared to habitual sleep. This effect size is comparable with the results from previous studies. It also lines up with the general findings from the meta-analysis by Schrimpf et al. (2015), where they report large effect sizes for within-subject pain experiments involving sleep deprivation. Similar to the results in this thesis, a within-group study by Tiede et al. (2010) reports a 30% increase in experimental pain ratings after sleep deprivation. However, a study by Matre et al. (2015) reports that only the highest intensity pain stimulus was affected by sleep condition, and that the effect was a mere 8% increase in perceived pain intensity.

Although there seems to be ample support for a general effect of sleep restriction-induced hyperalgesia, it is possible that the degree of increase in pain is dependent on type of stimuli. In the experiment by Tiede et al. (2010) laser evoked pain was used, while the study by Matre et al. (2015) used electrical pain stimulations. A meta-analysis by Lautenbacher et al. (2006) reports that the effect of sleep deprivation on pain perception was larger in experiments using pressure pain than in experiments using heat pain. They explain this effect with the type of nociceptors that are targeted by different kinds of pain stimuli. Pressure pain stimulates both superficial and deep tissue nociception. Pain stimuli like heat pain and electrical pain mainly target nociceptors in the superficial tissue of the skin. Deep tissue nociception could be influenced by alterations in the descending modulatory system to a higher degree than external nociceptors (Lautenbacher et al., 2006).

Static EEG

The analysis of the static EEG indices showed no significant changes in EEG activity after restricted sleep as compared to habitual sleep (Hypothesis 2, see “Aims, objectives and

hypotheses"). In other words, the mean level of activity within each of the frequency bands was similar in both sleep conditions.

This finding stands in contrast to previously published research on EEG correlates to pain and sleep deprivation. Correlations between theta activity and pain, as well as between alpha activity and pain have been suggested in previous studies (Nir et al., 2010; Gram et al., 2015; Camfferman et al., 2017; Jensen et al., 2013). Furthermore, decreased alpha activity and increased theta activity has been observed in studies looking into the effect of sleep deprivation (Kim et al., 2001; Strijkstra et al., 2003). It is possible that the lack of significant findings related to the alpha band in this study stems from the use of static EEG indices. One study on pain observes an initial increase in alpha activity, followed by a gradual decrease (Gram et al., 2015). When the dynamic EEG activity within a frequency band is reduced to a single mean, patterns like these could cancel out. Furthermore, this thesis has analyzed changes in absolute power, and not relative changes (i.e. the degree of change in one frequency band compared to the degree of change in the other frequency bands). This could account for the lack of findings regarding the static changes in the theta band. In the study by Jensen et al. (2013), changes in theta activity was only observed when analyzing the relative changes in power, and not when looking at absolute power.

Associations between pain and/or sleep, and the beta and gamma band are less established but have been observed in some experiments. Examples regarding the beta band include a pain related increase in beta activity in a study comparing patients with chronic pain to healthy volunteers (Stern et al., 2006). A similar pain related increase in beta activity was reported in an experimental pain study with healthy subjects (Gram et al., 2015). The study by Gram et al. (2015) also found gamma band activity to be associated with measures of chronic pain. Additionally, a study by Zhang et al. (2012) demonstrated that gamma band activity recorded over the somatosensory cortex predicted subjective pain intensity.

It is, however, important to note that most studies in the field of pain research either looks into the relationship between pain and sleep without the use of brain imaging, or studies EEG activity related to either pain or sleep deprivation. The combination of the three is less common. This means that direct comparison to other studies is challenging, as the present work represents a new approach to the topic of pain and sleep.

Another factor that could contribute to the dissimilarity in findings is the study samples. Results from work with clinical patients (e.g. Stern et al., 2006) are not directly comparable to those of healthy research volunteers (e.g. Kim et al., 2001; Gram et al., 2015). Similarly, sleep restriction in an experimental setting (e.g. Strijkstra et al., 2003) does not perfectly match up

with sleep deprivation caused by night shift work or insomnia. Furthermore, there are inconsistencies in how researchers define the different frequency bands. Some researchers subdivide the frequency bands into smaller ranges when analyzing their EEG data, i.e. beta1, beta2 and beta3 (e.g. Gram et al., 2015). This could partially explain why the static EEG indices in this thesis contradict earlier findings, as the association between EEG activity and pain could be more strongly related to a specific range within a frequency band. Additionally, some findings are specific to certain brain areas (e.g. Gram et al., 2015; Nir et al., 2010). An area-specific increase in activity could be invisible in the general EEG activity-trend if there was a similar decrease in another area. The static EEG analysis in this thesis is based on a mean calculated from activity spanning a two-minute period. It is likely that the activity within a frequency band could both increase and decrease during this time period. Though this thesis does not look into area-specific changes, an effort has been made to explore patterns of EEG activity occurring at a smaller time scale. The results from the dynamic analyses could pick up on changes in activity that are not reflected in the static EEG analysis.

Dynamic EEG

The change in pain intensity after restricted sleep was accompanied by changes in some, but not all, of the dynamic EEG indices (Hypothesis 4, see "Aims, objectives and hypotheses"). The dynamic analysis revealed that activity within the alpha band explained a significant amount of the variance in the VAS scores. Furthermore, activity in the theta band was associated with changes in VAS scores in the restricted sleep condition. For both the theta band and the alpha band, the relationship between EEG activity and pain was dependent on sleep condition. In the habitual sleep condition, *higher* pain ratings were associated with *higher* EEG activity. The opposite was true in the restricted sleep condition, where *lower* EEG activity in the theta and alpha band was associated with *higher* pain ratings.

Sleep deprived subjects have shown similar EEG characteristics in the alpha band as those of chronic pain patients (Camfferman et al., 2017). Several studies report a decrease in alpha activity after sleep deprivation (Kim et al., 2001; Strijkstra et al., 2003), and in relation to painful stimuli (Gram et al., 2015; Nir et al., 2010). A study by Camfferman et al. (2017) sought to distinguish the effect of sleep loss from that of pain on waking EEG activity. Their results indicate that both elevated pain intensity and poor sleep quality is related to a decrease in alpha activity, and more specifically, a decrease in the frontal and parietal areas of the cortex. The general trend of decreased alpha activity observed in Camfferman et al. (2017) are in line with this thesis' observation regarding alpha activity and pain after restricted sleep.

However, it contrasts the interaction effect seen in the habitual sleep condition, where an *increase* in alpha activity was related to higher pain scores. This finding seems to imply that lack of sleep can alter the way our brains processes pain, and that this might be reflected in changes occurring in the alpha rhythms. Some factors simply moderate the strength of a connection. Sleep might have a more impactful effect, changing the direction of the connection between pain perception and alpha waves.

Regarding the effect of the theta band, an increase in theta activity is found in patients suffering of neurogenic chronic pain (Llinás et al., 1999; Sarnthein et al., 2006; Stern et al., 2006). An experimental study on healthy subjects have on the other hand observed a *decrease* in theta activity when subjected to pain (Gram et al., 2015). While neither of these studies included sleep as variable, the theta rhythm nevertheless seems to be related to pain perception. In contrast to this thesis, the study by Camfferman et al. (2017) found no significant association between theta activity and pain, nor between theta activity and sleep quality. This could partially stem from differences in experimental design. Camfferman et al. (2017) gathered information on the subjects' sleep through a questionnaire on sleep length and ratings of sleep quality. Furthermore, the subjects suffered from chronic pain. In the present study, subjects came to the lab after two consecutive nights of habitual sleep and two consecutive nights at work, and none of the study subjects were affected by chronic pain disorders. Additionally, Camfferman et al. (2017) looked at correlations, while this thesis analyzed mean group differences between two distinct sleep conditions. More research is needed in order to determine whether this thesis' findings on theta activity are valid and can be reproduced in other samples.

When it comes to the delta, beta and gamma band, the present study observed no significant changes related to perceived pain intensity. The lack of significant findings associated with the delta band was not surprising, as there are very few studies reporting a connection between sleep, pain and activity within the delta band. The beta and gamma band, however, are more often implicated in experiments on sleep deprivation and pain perception (e.g. Gram et al., 2015; Stern et al., 2006). Regarding the beta band, the lack of findings could stem from the way the beta band is defined. The study by Gram et al. (2015), which reports a significant correlation between beta activity and pain, subdivides the beta band into three bands; beta1, beta2 and beta3. The beta3 band is the only beta band found to correlate with pain. Similarly, a study by Stern et al. (2006) reports changes in the lower range of the beta band. Their beta band is defined as activity occurring within the 12-25 Hz range. In the present study, the beta band was defined as activity occurring within the 12-32 Hz range. The

inconsistencies in how frequency bands are defined is most prominent when it comes to the beta and gamma bands (Babiloni, C. et al., 2020). It is thus likely that this might account for some of the differences in reported findings.

The lack of significant changes in the beta and gamma band could also be affected by the method of artifact removal, as some muscle artifacts still remained in the data after preprocessing (see Figure 9). The beta and gamma bands have been associated with artifacts from muscle contractions which can occur in the same frequency range as beta and gamma activity (Dowman et al., 2008). These artifacts, potentially originating from facial expressions related to pain, could have obscured pain-related changes in beta and gamma activity. A further expansion of the present study could employ additional artifact removal methods and subdivide the beta band prior to analysis. This could give more answers regarding the association between pain and beta and gamma activity.

Underlying mechanisms

The results from this thesis support the claim that sleep loss can alter pain perception. Which mechanisms that are involved does however remain unclear. Circling back to the introduction, one possible answer is through negative affect. Negative affect could also prove relevant for the observed changes in alpha activity in this thesis. The alpha band has repeatedly been linked with negative affect and depression, especially in prefrontal areas (Finn & Justus, 1999; Gollan et al., 2014;Zhang et al., 2019). Most studies investigating this matter focus on the asymmetry of the alpha EEG activity. For example, a hypoactivation of the alpha rhythm in the left frontal areas can be seen in subjects who were or had been depressed (Gollan et al., 2014). Zhang et al. (2019) propose that findings like these suggest that sleep deprivation could lead to a compromised emotional regulatory process. Furthermore, they hypothesize that activity within the alpha band could be involved in this process. However, there are also studies with contrasting findings, like an experiment by Gollan et al. (2014) which saw no changes in alpha activity during tasks meant to induce negative affect. A further expansion of the present study could give an indication as to whether subjects who are sleep deprived also have a hypoactivation of the alpha activity in frontal areas, or if it's specific to negative affect and depression.

Disregarding negative affect, some researchers attempt to explain the link between sleep and pain with inflammation. However, sleep loss has been linked empirically with both inflammation and negative affect. Instead of pitting these explanations up against each other, one might gain insights by investigating their interactions. A recent paper by Albrecht et al.

(2019) supports this notion, and states that a growing body of evidence associates neuroinflammation with both chronic pain and negative affect. This further suggests that inflammation could be a common substrate contributing to both conditions. Chronic pain is a multifaceted process and experience. It is unlikely that the causes and effects of pain are underpinned by a single straightforward mechanism. Rather, an interplay of biological events and cascades, such as sleep and inflammation, in combination with psychological concepts like negative affect is a more likely story.

Method discussion

The experiment that provided the data for this thesis was designed to investigate how night shift work affects pain sensitivity. More specifically, how sleep loss affects sensitivity to electrically induced pain, heat pain, cold pain, and pressure pain, as well as pain inhibition. Furthermore, the experiment was designed with event related analysis in mind. Other results from the experiment have already been published (Matre et al., 2017).

Overall, there is less pain research that use continuous EEG than there are studies utilizing event related EEG analysis. Analysis of continuous EEG data could be an important approach to use in order to uncover the mechanisms of chronic pain. Continuous EEG analysis was thus chosen as the focus of the present study despite the fact that the experiment was optimized for analysis of ERPs.

One of the shortcomings in the design is that the experiment lacks a control condition. Ideally, the experiment should have included a two-minute control condition with *pain-free* continuous EEG recordings in both the habitual sleep condition and in the restricted sleep condition. This would have made the dataset better suited for continuous EEG analysis on how sleep affects pain perception. The lack of a control condition makes it challenging to distinguish between the effect that sleep loss and pain has on waking EEG. There is a possibility that potential changes in activity within some of the bands could be of opposite characteristics and thereby offset one another, i.e. a sleep-related increase in activity and a similarly sized pain-related decrease in activity. The literature does however suggest that both sleep deprivation and pain have similar effects on alpha and theta activity. It is thus unlikely that sleep-related changes would hide the effect of pain, and vice versa, in these frequency bands.

A strength of the experimental design in this study is that it ensures that the study subjects come into the lab without having slept the night before. It is difficult to administer sleep restriction in studies where participants are instructed to go home and stay awake the whole

night, or sleep a particular number of hours. When the subjects are engaged at their work during the night, this is not an issue. The choice of study subjects also means that the generalizability of the results is currently limited to shift work, as the habitual sleep of shift workers could differ from the habitual sleep of non-shift workers. Studies indicate that shift workers may be prone to diseases relating to disrupted circadian rhythms (Baron & Reid, 2014; Haus & Smolensky, 2006; James et al., 2017) which could affect their habitual sleep. As the aim of the research project at STAMI is to investigate the health effects of shift work, the limitations of the generalizability are not a primary topic of concern.

Another strength of this study lies in the paired comparison of the data. With a within-subject design the participants act as their own control subjects. This means that real differences caused by the experimental conditions are less likely to remain undetected or covered up by random noise, diminishing the chances of a type II error.

When it comes to the specific methods employed in the preprocessing and analyses of the data, the method of artifact removal should be mentioned. Removing artifacts from the EEG signal was done manually by rejecting stretches of the signal which were highly characterized by artifacts. This mimics the method of Gram et al. (2015), where resting EEG was cleaned by selecting 2 minutes of artefact-free EEG from a 2-5 min recording (removing 20% of the signal). However, in some of the EEG recordings in the present study more than 20% of the signal had to be cut. This led to a nonuniform change in the length of the EEG signal for each subject. The static EEG analysis was not affected by this, but there were some consequences for the dynamic EEG analysis. When the EEG signal was divided into 15 second windows, the length of the signal dictated how many data points were made for each subject. This led to some challenges in how to compare individual subjects in the graphic representation of the data. The issue was handled by limiting the number of datapoints to be plotted per subject, stopping at the point where a large amount of the subjects no longer had any contributing data. Despite this issue, manual methods for artifact removal also have advantages. For example, it gives the researcher complete control over which parts of the data that is included in the final analysis. Future researchers dealing with continuous EEG data should consider the use of software that performs artifact removal through source separation and subtraction rather than rejection of data sections. However, software driven artifact removal can be complicated, and will most likely also lead to loosing parts of the signal. The use of artifact software was not prioritized during the present analysis, due to the extent of the thesis work.

A last point to mention is that the focus of this thesis has been on changes in global EEG activity, not area-specific activity. This is due to the timeline and the scope of the thesis, and

as a result of the number of electrodes used when recording EEG activity. Research indicates that one should use at least 35 electrodes, preferably more, in order to locate sources of the activity with a sufficient level of precision (Lau et al., 2012). As the cap used in this experiment had 32 electrodes, and two of them were used to track eye movements, the preciseness of source localization would not have been satisfactory. With this in mind, analysis of global EEG activity was deemed a better fit for this dataset than the analysis of area specific EEG.

Conclusion

The findings in this thesis strengthen the notion that sleep loss leads to a heightened sensation of pain. Additionally, analysis of dynamic EEG data indicated that both the theta band and the alpha band play a role in the connection between sleep and pain. No significant effects were identified for the other frequency bands. These findings contribute to the development of neurophysiological correlates of pain, both by confirming established findings, as well as by questioning the validity of others. We know that disruptions of sleep impacts pain perception, but we still have a fair way to go before we can fully understand the underlying mechanisms supporting this link. Negative affect and inflammation are two promising correlates of both pain and sleep that should be studied further.

References

Acharya, U. R., Faust, O., Kannathal, N., Chua, T., & Laxminarayan, S. (2005). Non-linear analysis of EEG signals at various sleep stages. *Computer Methods and Programs in Biomedicine*, *80*(1), 37–45. https://doi.org/10.1016/j.cmpb.2005.06.011

Åkerstedt, T., & Gillberg, M. (1990). Subjective and objective sleepiness in the active individual. *International Journal of Neuroscience*, *52*(1–2), 29–37. https://doi.org/10.3109/00207459008994241

Akin, M. (2002). Comparison of Wavelet Transform and FFT Methods in the Analysis of EEG Signals. *Journal of Medical Systems*, *26*(3), 241–247.

Albrecht, D. S., Kim, M., Akeju, O., Torrado-Carvajal, A., Edwards, R. R., Zhang, Y., Bergan, C., Protsenko, E., Kucyi, A., Wasan, A. D., Hooker, J. M., Napadow, V., & Loggia, M. L. (2019). The neuroinflammatory component of negative affect in patients with chronic pain. *Molecular Psychiatry*, 1–11. https://doi.org/10.1038/s41380-019-0433-1

Andersen, M. L., Araujo, P., Frange, C., & Tufik, S. (2018). Sleep Disturbance and Pain: A Tale of Two Common Problems. *Chest*, *154*(5), 1249–1259. https://doi.org/10.1016/j.chest.2018.07.019

Arnold, L. M., Choy, E., Clauw, D. J., Goldenberg, D. L., Harris, R. E., Helfenstein, M., Jensen, T. S., Noguchi, K., Silverman, S. L., Ushida, T., & Wang, G. (2016). Fibromyalgia and chronic pain syndromes: A white paper detailing current challenges in the field. *Clinical Journal of Pain*, *32*(9), 737–746. https://doi.org/10.1097/AJP.0000000000000354

Babiloni, A. H., De Koninck, B. P., Beetz, G., De Beaumont, L., Martel, M. O., & Lavigne, G. J. (2020). Sleep and pain: recent insights, mechanisms, and future directions in the investigation of this relationship. *Journal of Neural Transmission*, *127*(4), 647–660. https://doi.org/10.1007/s00702-019-02067-z

Babiloni, C., Barry, R. J., Başar, E., Blinowska, K. J., Cichocki, A., Drinkenburg, W. H., Klimesch, W., Knight, R. T., Lopes da Silva, F., Nunez, P., Oostenveld, R., Jeong, J., Pascual-Marqui, R., Valdes-Sosa, P., & Hallett, M. (2020). International Federation of Clinical Neurophysiology (IFCN) – EEG research workgroup: Recommendations on frequency and topographic analysis of resting state EEG rhythms. Part 1: Applications in clinical research studies. *Clinical Neurophysiology*, *131*(1), 285–307. https://doi.org/10.1016/j.clinph.2019.06.234

Baron, K. G., & Reid, K. J. (2014). Circadian misalignment and health. *International Review*

of Psychiatry, *26*(2), 139–154. https://doi.org/10.3109/09540261.2014.911149

Basbaum, A. I., Bautista, D. M., Scherrer, G., & Julius, D. (2009). *Cellular and Molecular Mechanisms of Pain Introduction: Acute Versus Persistent Pain*. 1–22. https://doi.org/10.1016/j.cell.2009.09.028

Basbaum, A. I., & Jessell, T. M. (2013). Part V: Perception, Chapter 24: Pain. In E. R. Kandel, T. M. Jessell, J. H. Schwartz, S. A. Siegelbaum, A. J. Hudspeth, & S. Mack (Eds.), *Principles of Neural Science* (pp. 530–555). McGraw-Hill Medical.

Basner, M., & Dinges, D. F. (2011). Maximizing sensitivity of the Psychomotor Vigilance Test (PVT) to sleep loss. *Sleep*, *34*(5), 581–591. https://doi.org/10.1093/sleep/34.5.581

Bassani, T., & Nievola, J. C. (2008). Pattern recognition for brain-computer interface on disabled subjects using a wavelet transformation. *IEEE Symposium on Computational Intelligence in Bioinformatics and Computational Biology, CIBCB '08*, 180–186. https://doi.org/10.1109/CIBCB.2008.4675776

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

Bergman, S., Herrström, P., Högström, K., Petersson, I. F., Svensson, B., & Jacobsson, L. T. H. (2001). Chronic musculoskeletal pain, prevalence rates, and sociodemographic associations in a Swedish population study. *Journal of Rheumatology*, *28*(6), 1369–1377.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain*, *10*(4), 287–287. https://doi.org/10.1016/j.ejpain.2005.06.009

Camfferman, D., Moseley, G. L., Gertz, K., Pettet, M. W., & Jensen, M. P. (2017). Waking EEG Cortical Markers of Chronic Pain and Sleepiness. *Pain Medicine (United States)*, *18*(10), 1921–1931. https://doi.org/10.1093/pm/pnw294

Chapter 01: Rejecting Artifacts. (2019). https://sccn.ucsd.edu/wiki/Chapter_01:_Rejecting_Artifacts

Chen, J. (2011). History of pain theories. *Neurosci Bull*, *27*(5), 343–350. https://doi.org/10.1007/s12264-011-0139-0

Damien, J., Colloca, L., Bellei-Rodriguez, C. É., & Marchand, S. (2018). Pain Modulation: From Conditioned Pain Modulation to Placebo and Nocebo Effects in Experimental and Clinical Pain. In *International Review of Neurobiology* (Vol. 139, pp. 255–296). Academic Press. https://doi.org/10.1016/bs.irn.2018.07.024

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-

trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*, 9–21. http://www.sccn.ucsd.edu/eeglab/

Do-Won, K., & Chang-Hwan, I. (2018). EEG Spectral Analysis. In I. Chang-Hwan (Ed.), *Computational EEG Analysis: Methods and Applications* (pp. 35–53). Springer Singapore. https://doi.org/10.1007/978-981-13-0908-3_3

dos Santos Pinheiro, E. S., de Queirós, F. C., Montoya, P., Santos, C. L., do Nascimento, M. A., Ito, C. H., Silva, M., Santos, D. B. N., Benevides, S., Miranda, J. G. V., Sá, K. N., & Baptista, A. F. (2016). Electroencephalographic patterns in chronic pain: A systematic review of the literature. *PLoS ONE*, *11*(2). https://doi.org/10.1371/journal.pone.0149085

Dowman, R., Rissacher, D., & Schuckers, S. (2008). EEG indices of tonic pain-related activity in the somatosensory cortices. *Clinical Neurophysiology*, *119*(5), 1201–1212. https://doi.org/10.1016/j.clinph.2008.01.019

Engel, A. K., & Fries, P. (2010). Beta-band oscillations-signalling the status quo? *Current Opinion in Neurobiology*, *20*(2), 156–165. https://doi.org/10.1016/j.conb.2010.02.015

Evans, J. (2007). *Your Psychology Project: The Essential Guide*. SAGE Publications, Ltd.

Finan, P. H., Goodin, B. R., & Smith, M. T. (2013). The association of sleep and pain: An update and a path forward. *Journal of Pain*, *14*(12), 1539–1552. https://doi.org/10.1016/j.jpain.2013.08.007

Finn, P. R., & Justus, A. (1999). Reduced EEG Alpha Power in the Male and Female Offspring of Alcoholics. *Alcoholism: Clinical and Experimental Research*, *23*(2), 256–262. https://doi.org/10.1111/j.1530-0277.1999.tb04108.x

Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2007). The Biopsychosocial Approach to Chronic Pain: Scientific Advances and Future Directions. *Psychological Bulletin*, *133*(4). https://doi.org/10.1037/0033-2909.133.4.581

Gollan, J. K., Hoxha, D., Chihade, D., Pflieger, M. E., Rosebrock, L., & Cacioppo, J. (2014). Frontal alpha EEG asymmetry before and after behavioral activation treatment for depression. *Biological Psychology*, *99*(1), 198–208. https://doi.org/10.1016/j.biopsycho.2014.03.003

Gram, M., Graversen, C., Olesen, S. S., & Drewes, A. M. (2015). Dynamic spectral indices of the electroencephalogram provide new insights into tonic pain. *Clinical Neurophysiology*, *126*(4), 763–771. https://doi.org/10.1016/j.clinph.2014.07.027

Haack, M., Scott-Sutherland, J., Santangelo, G., Simpson, N. S., Sethna, N., & Mullington, J. M. (2012). Pain sensitivity and modulation in primary insomnia. *European Journal of Pain*, *16*(4), 522–533. https://doi.org/10.1016/j.ejpain.2011.07.007

Haack, Monika, Lee, E., Cohen, D. A., & Mullington, J. M. (2009). Activation of the prostaglandin system in response to sleep loss in healthy humans: Potential mediator of increased spontaneous pain. *Pain*, *145*(1–2), 136–141. https://doi.org/10.1016/j.pain.2009.05.029

Haefeli, M., & Elfering, A. (2006). Pain assessment. *European Spine Journal*, *15*(1), 17–24. https://doi.org/10.1007/s00586-005-1044-x

Hanssen, M. M., Peters, M. L., Boselie, J. J., & Meulders, A. (2017). Can positive affect attenuate (persistent) pain? State of the art and clinical implications. *Current Rheumatology Reports*, *19*(12), 1–9. https://doi.org/10.1007/s11926-017-0703-3

Haus, E., & Smolensky, M. (2006). Biological clocks and shift work: Circadian dysregulation and potential long-term effects. *Cancer Causes and Control*, *17*(4), 489–500. https://doi.org/10.1007/s10552-005-9015-4

Hollis, S., & Campbell, F. (1999). What is meant by intention to treat analysis? Survey of published randomised controlled trials. *British Medical Journal*, *319*(7211), 670–674. https://doi.org/10.1136/bmj.319.7211.670

IASP Task Force. (2012). Classification of Chronic Pain, Second Edition (Revised). In *IASP Press.*

James, S. M., Honn, K. A., Gaddameedhi, S., & Van Dongen, H. P. (2017). Shift Work: Disrupted Circadian Rhythms and Sleep—Implications for Health and Well-being. *Current Sleep Medicine Reports*, *3*(2), 104–112. https://doi.org/10.1007/s40675-017-0071-6

Jensen, M. P., Sherlin, L. H., Gertz, K. J., Braden, A. L., Kupper, A. E., Gianas, A., Howe, J. D., & Hakimian, S. (2013). Brain EEG activity correlates of chronic pain in persons with spinal cord injury: Clinical implications. *Spinal Cord*, *51*(1), 55–58. https://doi.org/10.1038/sc.2012.84

Kahana, M. J. (2006). The Cognitive Correlates of Human Brain Oscillations. *Journal of Neuroscience*, *26*(6), 1669–1672. https://doi.org/10.1523/JNEUROSCI.3737-05c.2006

Kaila-Kangas, L., Kivimäki, M., Härmä, M., Riihimäki, H., Luukkonen, R., Kirjonen, J., & Leino-Arjas, P. (2006). Sleep Disturbances as Predictors of Hospitalization for Back Disorders–A 28-Year Follow-up of Industrial Employees. *Spine*, *31*(1), 51–56. https://doi.org/10.1097/01.brs.0000193902.45315.e5

Kandel, E. R., Jessell, T. M., Schwartz, J. H., Siegelbaum, S. A., Hudspeth, A. J., & Mack, S. (Eds.). (2013). *Principles of Neural Science, Fifth Edition*. McGraw-Hill Medical.

Kecklund, G., & Axelsson, J. (2016). Health consequences of shift work and insufficient

sleep. *BMJ*, *355*, i5210. https://doi.org/10.1136/bmj.i5210

Kim, H., Guilleminault, C., Hong, S., Kim, D., Kim, S., Go, H., & Lee, S. (2001). Pattern analysis of sleep-deprived human EEG. *Journal of Sleep Research*, *10*(3), 193–201. https://doi.org/10.1046/j.1365-2869.2001.00258.x

Klimesch, W. (1997). EEG-alpha rhythms and memory processes. *International Journal of Psychophysiology*, *26*(1–3), 319–340. https://doi.org/10.1016/S0167-8760(97)00773-3

Knyazev, G. G. (2012). EEG delta oscillations as a correlate of basic homeostatic and motivational processes. *Neuroscience and Biobehavioral Reviews*, *36*(1), 677–695. https://doi.org/10.1016/j.neubiorev.2011.10.002

Konjarski, M., Murray, G., Lee, V. V., & Jackson, M. L. (2018). Reciprocal relationships between daily sleep and mood: A systematic review of naturalistic prospective studies. *Sleep Medicine Reviews*, *42*. https://doi.org/10.1016/j.smrv.2018.05.005

Krause, A. J., Prather, A. A., Wager, T. D., Lindquist, M. A., & Walker, M. P. (2019). The pain of sleep loss: A brain characterization in humans. *Journal of Neuroscience*, *39*(12), 2291–2300. https://doi.org/10.1523/JNEUROSCI.2408-18.2018

Krueger, J. M., Clinton, J. M., Winters, B. D., Zielinski, M. R., Taishi, P., Jewett, K. A., & Davis, C. J. (2011). Involvement of cytokines in slow wave sleep. In *Progress in Brain Research* (Vol. 193, pp. 39–47). Elsevier. https://doi.org/10.1016/B978-0-444-53839-0.00003-X

Kundermann, B., Spernal, J., Huber, M. T., Krieg, J.-C., & Lautenbacher, S. (2004). Sleep Deprivation Affects Thermal Pain Thresholds but Not Somatosensory Thresholds in Healthy Volunteers. *Psychosomatic Medicine*, *66*(6), 932–937. https://doi.org/10.1097/01.psy.0000145912.24553.c0

Landmark, T., Dale, O., Romundstad, P., Woodhouse, A., Kaasa, S., & Borchgrevink, P. C. (2018). Development and course of chronic pain over 4 years in the general population: The HUNT pain study. *European Journal of Pain*, *22*(9), 1606–1616. https://doi.org/10.1002/ejp.1243

Lau, T. M., Gwin, J. T., & Ferris, D. P. (2012). How Many Electrodes Are Really Needed for EEG-Based Mobile Brain Imaging? *Journal of Behavioral and Brain Science*. https://doi.org/10.4236/jbbs.2012.23044

Lautenbacher, S., Kundermann, B., & Krieg, J.-C. (2006). Sleep deprivation and pain perception. *Sleep Medicine Reviews*, *10*, 357–369. https://doi.org/10.1016/j.smrv.2005.08.001

Lentz, M. J., Landis, C. A., Rothermel, J., & Shaver, J. L. F. (1999). Effects of selective slow

wave sleep disruption on musculoskeletal pain and fatigue in middle aged women. *Journal of Rheumatology*, *26*(7), 1586–1592.

Llinás, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., & Mitra, P. P. (1999). Thalamocortical dysrhythmia: A neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proceedings of the National Academy of Sciences*, *96*(26), 15222–15227. https://doi.org/10.1073/pnas.96.26.15222

Louis, E. K. S., & Frey, L. C. (2016). Electroencephaolgraphy - An introductory text. In *Electroencephalography (EEG): An Introductory Text and Atlas of Normal and Abnormal Findings in Adults, Children, and Infants*. American Epilepsy Society.

Matre, D., Hu, L., Viken, L. A., Hjelle, I. B., Wigemyr, M., Trond, S., Knardahl, S., Nilsen, K. B., Sand, T., & Nilsen, K. B. (2015). Experimental Sleep Restriction Facilitates Pain and Electrically Induced Cortical Responses. *SLEEP*, *38*(10), 1607–1617. https://doi.org/10.5665/sleep.5058

Matre, D., Knardahl, S., & Nilsen, K. B. (2017). Night-shift work is associated with increased pain perception. *Scandinavian Journal of Work, Environment and Health*, *43*(3), 260–268. https://doi.org/10.5271/sjweh.3627

Melzack, R., & Casey, K. L. (1968). Sensory, Motivational, and Central Control Determinants of Pain: A New Conceptual Model. In *The Skin Senses: Proceedings of the First International Symposium on the Skin Senses*. https://doi.org/10.1108/QMR-12-2012-0057

Mills, S. E. E., Nicolson, K. P., & Smith, B. H. (2019). Chronic pain: a review of its epidemiology and associated factors in population-based studies. *British Journal of Anaesthesia*, *123*(2), e273–e283. https://doi.org/10.1016/j.bja.2019.03.023

Mitra, P., & Bokil, H. (2009). *Observed Brain Dynamics*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195178081.001.0001

Moayedi, M., & Davis, K. D. (2013). Theories of pain: From specificity to gate control. *Journal of Neurophysiology*, *109*(1), 5–12. https://doi.org/10.1152/jn.00457.2012

Morin, C. M., Bélanger, L., LeBlanc, M., Ivers, H., Savard, J., Espie, C. A., Mérette, C., Baillargeon, L., & Grégoire, J. P. (2009). The natural history of insomnia a population-based 3-year longitudinal study. *Archives of Internal Medicine*, *169*(5), 447–453. https://doi.org/10.1001/archinternmed.2008.610

Mork, P. J., & Nilsen, T. I. (2012). Sleep problems and risk of fibromyalgia: Longitudinal data on an adult female population in Norway. *Arthritis & Rheumatism*, *64*(1), 281–284. https://doi.org/10.1002/art.33346

Morton, D. L., Sandhu, J. S., & Jones, A. K. P. (2016). Brain imaging of pain: State of the art. *Journal of Pain Research*, *9*, 613–624. https://doi.org/10.2147/JPR.S60433

Nicholas, M., Vlaeyen, J. W. S., Rief, W., Barke, A., Aziz, Q., Benoliel, R., Cohen, M., Evers, S., Giamberardino, M. A., Goebel, A., Korwisi, B., Perrot, S., Svensson, P., Wang, S.-J., & Treede, R.-D. (2019). The IASP classification of chronic pain for ICD-11. *PAIN*, *160*(1), 28–37. https://doi.org/10.1097/j.pain.0000000000001390

Nir, R. R., Sinai, A., Raz, E., Sprecher, E., & Yarnitsky, D. (2010). Pain assessment by continuous EEG: Association between subjective perception of tonic pain and peak frequency of alpha oscillations during stimulation and at rest. *Brain Research*, *1344*, 77–86. https://doi.org/10.1016/j.brainres.2010.05.004

Nowack, W. J. (1995). Neocortical dynamics and human eeg rhythms. *Neurology*, *45*(9), 1793. https://doi.org/10.1212/WNL.45.9.1793-a

Ødegård, S. S., Engstrøm, M., Sand, T., Stovner, L. J., Zwart, J. A., & Hagen, K. (2010). Associations between sleep disturbance and primary headaches: the third Nord-Trøndelag Health Study. *The Journal of Headache and Pain*, *11*(3), 197–206. https://doi.org/10.1007/s10194-010-0201-8

Orzeł-Gryglewska, J. (2010). CONSEQUENCES OF SLEEP DEPRIVATION. *International Journal of Occupational Medicine and Environmental Health*, *23*(1), 95–114. https://doi.org/10.2478/v10001-010-0004-9

Peng, W., & Tang, D. (2015). Pain related cortical oscillations: Methodological advances and potential applications. *Frontiers in Computational Neuroscience*, *10*. https://doi.org/10.3389/fncom.2016.00009

Ploner, M., Sorg, C., & Gross, J. (2017). Brain Rhythms of Pain. *Trends in Cognitive Sciences*, *21*(2), 100–110. https://doi.org/10.1016/j.tics.2016.12.001

Porkka-Heiskanen, T. (2013). Sleep homeostasis. *Current Opinion in Neurobiology*, *23*(5), 799–805. https://doi.org/10.1016/j.conb.2013.02.010

Potthoff, R. F., Tudor, G. E., Pieper, K. S., & Hasselblad, V. (2006). Can one assess whether missing data are missing at random in medical studies? *Statistical Methods in Medical Research*, *15*(3), 213–234. https://doi.org/10.1191/0962280206sm448oa

Saper, C. B., Fuller, P. M., Pedersen, N. P., Lu, J., & Scammell, T. E. (2010). Sleep State Switching. *Neuron*, *68*(6), 1023–1042. https://doi.org/10.1016/j.neuron.2010.11.032

Sarnthein, J., Stern, J., Aufenberg, C., Rousson, V., & Jeanmonod, D. (2006). Increased EEG power and slowed dominant frequency in patients with neurogenic pain. *Brain*, *129*, 55–64. https://doi.org/10.1093/brain/awh631

Sateia, M. J. (2014). International Classification of Sleep Disorders - Third Edition highlights and modifications. *Chest*, *146*(5), 1387–1394. https://doi.org/10.1378/chest.14-0970

Schrimpf, M., Liegl, G., Boeckle, M., Leitner, A., Geisler, P., & Pieh, C. (2015). The effect of sleep deprivation on pain perception in healthy subjects: a meta-analysis. *Sleep Medicine*, *16*(11), 1313–1320. https://doi.org/10.1016/j.sleep.2015.07.022

Schuh-Hofer, S., Wodarski, R., Pfau, D. B., Caspani, O., Magerl, W., Kennedy, J. D., & Treede, R. D. (2013). One night of total sleep deprivation promotes a state of generalized hyperalgesia: A surrogate pain model to study the relationship of insomnia and pain. *Pain*, *154*(9), 1613–1621. https://doi.org/10.1016/j.pain.2013.04.046

Schulz, E., Tiemann, L., Schuster, T., Gross, J., & Ploner, M. (2011). Neurophysiological Coding of Traits and States in the Perception of Pain. *Cerebral Cortex*, *21*(10), 2408–2414. https://doi.org/10.1093/cercor/bhr027

Staud, R., Cannon, R. C., Mauderli, A. P., Robinson, M. E., Price, D. D., & Vierck, C. J. (2003). Temporal summation of pain from mechanical stimulation of muscle tissue in normal controls and subjects with fibromyalgia syndrome. *Pain*, *102*(1–2), 87–95. https://doi.org/10.1016/s0304-3959(02)00344-5

Stern, J., Jeanmonod, D., & Sarnthein, J. (2006). Persistent EEG overactivation in the cortical pain matrix of neurogenic pain patients. *NeuroImage*, *31*(2), 721–731. https://doi.org/10.1016/j.neuroimage.2005.12.042

Strijkstra, A. M., Beersma, D. G. M., Drayer, B., Halbesma, N., & Daan, S. (2003). Subjective sleepiness correlates negatively with global alpha (8-12 Hz) and positively with central frontal theta (4-8 Hz) frequencies in the human resting awake electroencephalogram. *Neuroscience Letters*, *340*(1), 17–20. https://doi.org/10.1016/S0304-3940(03)00033-8

Tiede, W., Magerl, W., Baumgärtner, U., Durrer, B., Ehlert, U., & Treede, R. D. (2010). Sleep restriction attenuates amplitudes and attentional modulation of pain-related evoked potentials, but augments pain ratings in healthy volunteers. *Pain*, *148*(1), 36–42. https://doi.org/10.1016/j.pain.2009.08.029

Tononi, G., & Cirelli, C. (2006). Sleep function and synaptic homeostasis. *Sleep Medicine Reviews*, *10*(1), 49–62. https://doi.org/10.1016/j.smrv.2005.05.002

Torrence, C., & Compo, G. P. (1998). A Practical Guide to Wavelet Analysis. *Bulletin of the American Meteorological Society*, *79*(1), 61–78. https://doi.org/10.1175/1520-0477(1998)079<0061:APGTWA>2.0.CO;2

Tracey, I. (2008). Imaging pain. *British Journal of Anaesthesia*, *101*(1).

https://doi.org/10.1093/bja/aen102

Trinkoff, A. M., Le, R., Geiger-Brown, J., Lipscomb, J., & Lang, G. (2006). Longitudinal relationship of work hours, mandatory overtime, and on-call to musculoskeletal problems in nurses. *American Journal of Industrial Medicine*, *49*(11), 964–971. https://doi.org/10.1002/ajim.20330

Uhlig, B. L., Sand, T., Nilsen, T. I., Mork, P. J., & Hagen, K. (2018). Insomnia and risk of chronic musculoskeletal complaints: Longitudinal data from the HUNT study, Norway. *BMC Musculoskeletal Disorders*, *19*(1). https://doi.org/10.1186/s12891-018-2035-5

Uhlig, B. L., Sand, T., Ødegård, S. S., & Hagen, K. (2014). Prevalence and associated factors of DSM-V insomnia in Norway: The Nord-Trøndelag Health Study (HUNT 3). *Sleep Medicine*, *15*(6), 708–713. https://doi.org/10.1016/j.sleep.2014.01.018

van der Miesen, M. M., Lindquist, M. A., & Wager, T. D. (2019). Neuroimaging-based biomarkers for pain. *PAIN Reports*, *4*(4), e751. https://doi.org/10.1097/pr9.0000000000000751

Verbeke, G. (1997). Linear Mixed Models for Longitudinal Data. In *In Linear mixed models in practice* (pp. 63–153). Springer. https://doi.org/10.1007/978-1-4612-2294-1_3

Vierck, C. J., Cannon, R. L., Fry, G., Maixner, W., & Whitsel, B. L. (1997). Characteristics of Temporal Summation of Second Pain Sensations Elicited by Brief Contact of Glabrous Skin by a Preheated Thermode. *Journal of Neurophysiology*, *78*(2), 992–1002. https://doi.org/10.1152/jn.1997.78.2.992

Vos, T., Abajobir, A. A., Abbafati, C., Abbas, K. M., Abate, K. H., Abd-Allah, F., Abdulle, A. M., Abebo, T. A., Abera, S. F., Aboyans, V., Abu-Raddad, L. J., Ackerman, I. N., Adamu, A. A., Adetokunboh, O., Afarideh, M., Afshin, A., Agarwal, S. K., Aggarwal, R., Agrawal, A., … Murray, C. J. L. (2017). Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet*, *390*(10100), 1211–1259. https://doi.org/10.1016/S0140-6736(17)32154-2

Xu, X., & Huang, Y. (2020). Objective Pain Assessment: a Key for the Management of Chronic Pain. *F1000Research*, *9*. https://doi.org/10.12688/f1000research.20441.1

Yarnitsky, D. (2015). Role of endogenous pain modulation in chronic pain mechanisms and treatment. *PAIN*, *156*(4), 24–31. https://doi.org/10.1097/01.j.pain.0000460343.46847.58

Zhang, J., Lau, E. Y. Y., & Hsiao, J. H. (2019). Sleep deprivation compromises resting-state emotional regulatory processes: An EEG study. *Journal of Sleep Research*, *28*(3),

e12671. https://doi.org/10.1111/jsr.12671

Zhang, Z. G., Hu, L., Hung, Y. S., Mouraux, A., & Iannetti, G. D. (2012). Gamma-band oscillations in the primary somatosensory cortex-A direct and obligatory correlate of subjective pain intensity. *Journal of Neuroscience*, *32*(22), 7429–7438. https://doi.org/10.1523/JNEUROSCI.5877-11.2012

Zhao, I., Bogossian, F., & Turner, C. (2012). The Effects of Shift Work and Interaction Between Shift Work and Overweight/Obesity on Low Back Pain in Nurses. *Journal of Occupational and Environmental Medicine*, *54*(7), 820–825. https://doi.org/10.1097/jom.0b013e3182572e6a

Appendix

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Appendix I: Liner Mixed Model summaries of dynamic EEG analyses

Appendix A
Health form

Statens arbeidsmiljøinstitutt

Kjære forsøksdeltaker

Vi søker i dette prosjektet etter friske forsøkspersoner mellom 18 og 45 år. Hensikten med dette skjemaet er å kartlegge helsesituasjonen til forsøksdeltakerne. I tillegg ønsker vi å kartlegge noen andre faktorer som har betydning for smertefysiologiske forsøk. Vi ber deg om å svare på alle spørsmålene og returnere skjemaet ved å poste det i utlevert konvolutt.

1. Hvor gammel er du?	**25**	
2. Kjønn	Kvinne	Mann
Sett et kryss i kolonnene til høyre for hvert spørsmål	**Ja**	**Nei**
3. Er du frisk?		
4. Har du hatt vedvarende (mer enn 3 mnd) smerter i noen del av kroppen de siste 2 årene?		
5. Hvis du svarte ja på spørsmålet over, hvor sterke var disse smertene på en skala fra 0 til 10, hvor 0 er ingen smerte og 10 er verst tenkelig smerte?		
6. Har du hatt, eller har, en sykdom i en av følgende kategorier:		
a. Psykiatrisk sykdom (angst, depresjon inkludert)		
b. Nevrologisk sykdom		
c. Hjertesykdom		
d. Lungesykdom (velregulert astma er lov)		
7. Har du hodepine 2 dager eller mer pr. måned (i gjennomsnitt)		
8. Hvis du av og til har hodepine, hvor sterk er hodepinen du vanligvis har:		
a. Mild		
b. Moderat		
c. Kraftig		
9. Bruker du noen form for medisiner fast (inkludert håndkjøpsanalgetika som paracet/ibux)?		
Hvis ja, hvilken type:		
10. Har du høyt blodtrykk (mer enn 140/90 mmHg)?		
Vet ikke		

Se også baksiden

11. Er du gravid?		
12. Ammer du?		
13. Har du reagert med overfølsomhet for elektrodepasta eller saltholdige kremer tidligere?		
14. Jobber du skiftarbeid med nattevakter? Spesifiser på neste side		
15. Har du en diagnostisert søvnlidelse (eks. obstruktiv søvnapne, insomni, essensiell hypersomni, narkolepsi)		
Hvis ja, hvilken:		
16. For kvinner: Dato for siste menstruasjons første dag		

*Vi gjør oppmerksom på at du ikke må være **alkoholpåvirket** de siste 24 t før hver forsøksdag. Vi ber deg også om å avstå fra **kaffe, te og røyk/snus** siste time før du møter til undersøkelsen.*

Skiftarbeid

Jobber du aldri nattevakter? _____

Jobber du faste nattevakter? _____

Jobber du av og til nattevakter (ekstravakter)? _____ Hvis du svarte ja på en av de to siste spørsmålene, vennligst skisser vaktplanen for de siste to måneder nedenfor.

Appendix B

Information and consent form

Forespørsel om deltakelse i forskningsprosjektet

"Skiftarbeid og smertefølsomhet"

Bakgrunn og hensikt
Dette er et spørsmål til deg om å delta i en forskningsstudie hvor formålet er å bestemme om skiftarbeid fører til ulike helseplager. Personer som ikke jobber skift [ ] og personer som jobber varierende dag- og nattskift [ ] blir spurt om å delta.

Skiftarbeid kan være ugunstig for helsa. Vi vet i dag for lite om eventuelle mekanismer for dette og det er bakgrunnen for at Statens arbeidsmiljøinstitutt (STAMI) har planlagt denne studien.

Hva innebærer studien?
Studien innebærer deltakelse i tre laboratorieforsøk ved STAMI, samt registrering av søvn to døgn i forkant av hvert disse forsøkene. Det første laboratorieforsøket foregår i forbindelse med montering av søvnmålerutstyret og varer i ca 1,5 time. De to andre laboratorieforsøkene foregår morgenen etter siste søvnregistrering og varer i ca 2,5 timer. To dager før laboratorieforsøk nr 2 må du også møte på STAMI ca en halvtime for å få påmontert søvnmålerutstyr. Personer som ikke jobber skift vil bli bedt om å redusere sin normale søvnlengde i en eller begge nettene forut for et av forsøkene. Personer som jobber skift deltar i de samme laboratorieforsøkene etter siste nattevakt i en serie av påfølgende nattevakter og etter minst 3 påfølgende dagvakter. Registrering av søvn skjer ved ustyr som registrerer bevegelser og/eller søvnmønster. Man sover hjemme som normalt. Montering av utstyret skjer ved STAMI 2 døgn før hvert laboratorieforsøk.

Under laboratorieforsøkene vil det gjennomføres flere nevrofysiologiske tester. Et eksempel på en slik test er trykk mot huden. Noen stimuleringer kan være smertefulle. De nevrofysiologiske testene vil utføres flere steder på kroppen. De fleste testene er av kort varighet (få sekunder), mens noen varer i 5-6 minutter. De korteste testene gjentas evt. flere ganger. En deltaker kan når som helst be om at testene avbrytes. Under testene er det innlagt flere pauser. Testene er beskrevet i vedlegg A. Som deltaker vil du bli bedt om å vurdere intensiteten til stimuleringene vha. en skala. Under enkelte av testene vil hjerteaktivitet (EKG), blodtrykk, svetterespons og den elektriske aktiviteten fra hjernen (EEG) registreres.

Mulige fordeler og ulemper
Deltakelse i studien vil ikke gi noen personlige fordeler. Erfaringene fra studien vil imidlertid kunne bidra til bedre kartlegging av risikofaktorer for å utvikle kroniske smerter og kunnskap om planlegging av skiftordninger som er mindre helseskadelige. Andre fordeler kan være redusert sykefravær. Deltakelse i studien vil ikke medføre andre ulemper enn at de deltakerne som ikke jobber skift får mindre søvn forut for en av undersøkelsene.

Hva skjer med informasjonen om deg?
Informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres

Frivillig deltakelse
Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke

til å delta i studien. Dette vil ikke få noen konsekvenser. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte forsker, ph.d. Dagfinn Matre, tlf 23 19 51 00.

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*
Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Kriterier for deltakelse
For å delta i studien må du være mellom 18 og 60 år og forstå norsk muntlig og skriftlig. Du kan ikke delta dersom du har kroniske smerter (mer enn 3 måneder i løpet av siste 2 år), er avhengig av narkotika, er gravid, har psykiatrisk sykdom, har nevrologisk sykdom (mild hodepine 1 - 2 dager per måned er tillatt), har høyt blodtrykk, har kreft, eller bruker medikamenter mot epilepsi, depresjon eller nevrologiske lidelser funksjon.

Laboratorieforsøk

Nevrofysiologiske tester

Laboratorietestene ved STAMI vil bestå av følgende tester. I de fleste testene blir du bedt om å bestemme intensiteten til hver enkelt stimulering.

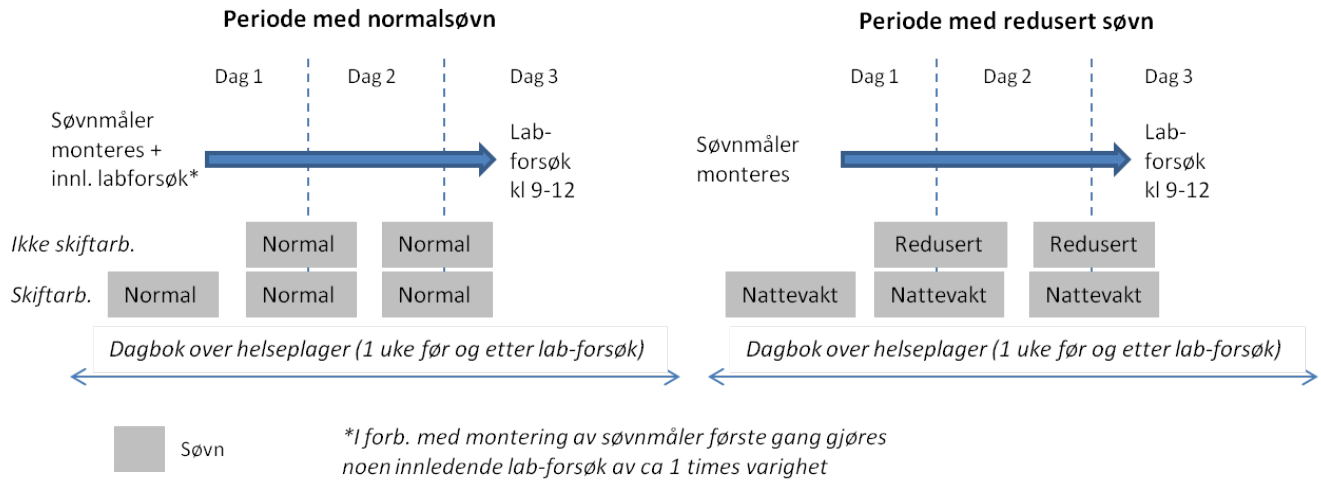
Del	Test[1]	Beskrivelse
1	**Smerteterskler** • Trykk • Varme • Kulde • Elektrisk	Smerteterskler bestemmes ved at ved at intensiteten på stimuleringen gradvis økes inntil moderat smerte kjennes og testen avbrytes. Gjentas 2-3 ganger for hver type stimulering.
	EEG monteres	En hette med 32 elektroder plasseres på hodet. Litt gele sprøytes i hver elektrode slik at vi kan registrere den elektriske aktiviteten fra hjernen.
2	**Elektrisk stimulering** • 3 x 30 elektriske stimuleringer.	Gjennom to elektroder klistret på armen sendes elektrisk strøm (1-5 mA). Hver elektrisk stimulering er veldig kort (noen millisekunder) og oppleves som et lite nålestikk mot huden.
3	**Spørreskjema**	Hver forsøksdag vil du bli bedt om å svare på et spørreskjema om helseplager.
4	**Varmestimulering + smerte på motsatt arm** • Varmestim • Varmestim + smerte på motsatt arm	Et varmelegeme legges inntil huden på armen og varmes opp til du kjenner moderat smerte. Dette gjentas 3-5 ganger. Varmelegemet ligger inntil huden i 2 min. Disse varmetestene gjentas etter smertefull stimulering på motsatt arm.
	EEG avmonteres	EEG-hetten tas av og du får mulighet til å vaske håret med sjampo.

[1]Nøyaktig rekkefølge og antall tester kan avvike noe fra det som er beskrevet her. EEG = elektroencephalografi (registrering av hjernens elektriske aktivitet).

Søvnmåling
Søvn registreres i 2 døgn før hver laboratorietest og montering av søvnmåler gjøres ved STAMI eller på din arbeidsplass om morgenen 2 dager før. Søvnmåleren består av registreringsenhet på størrelse med et armbåndsur og festes med en reim til ankel, håndledd eller overarm. Søvnmåleren tas av før lab-forsøket dag 3.

Dagbok
Mellom dag 1 og i en uke etter dag 3 vil du bli bedt om å fylle ut et skjema over hvilke helseplager du har hatt den dagen. Skjemaet vil fylles ut på papir, via internett eller via mobiltelefon.

Tidsskjema
Deltakelse i studien går over to perioder, en periode med normal søvn og en med redusert søvn. For deltakere som ikke jobber skift innebærer perioden med redusert søvn f.eks at du blir bedt om å sove halvparten av din normale nattesøvn de siste to nettene før et av lab-forsøkene. Noen deltakere vil bli bedt om å avstå fra søvn en natt. For deltakere som jobber skift vil perioden med redusert søvn være perioden med tre påfølgende nattevakter.



Mulige bivirkninger
Ved elektrisk- og varmestimulering som beskrevet i dette prosjektet blir huden av og til rød som ved solbrenthet. Dette vil være over i løpet av noen døgn og vil ikke gi noen varige skader. Huden i dette området kan også bli noe overfølsom for berøring, noe som varer maksimalt i noen timer. Det er lite sannsynlig at du vil hemmes av denne overfølsomheten. Ellers er det ikke rapportert noen kjente bivirkninger.

Fordeler og ulemper ved deltakelse
Studien innebærer ingen personlige fordeler ut over en økonomisk kompensasjon for å dekke tapt arbeidsfortjeneste og utgifter til transport. Ulempene ved å delta er knyttet til følgene av redusert søvn, samt laboratorietestene som innebærer noe smerte. Denne smerten er av en slik art at den ikke skader kroppen, men kun gir et relativt kortvarig ubehag.

Eventuell kompensasjon til og dekning av utgifter for deltakere
Det gis en kompensasjon på 150 kr/time til deltakerne for tidsbruk. Tidsbruk ved labforsøket dag 1 (første gang) anslås til ca 1,5 time. Tidsbruk ved labforsøket dag 2 og 3 anslås til ca 2,5 timer hver gang. I tillegg dekkes reisekostnader med offentlig transport til/fra STAMI t.o.m. Ruters sone 4 (ruter.no). Godtgjørelsen blir utbetalt 2-3 uker etter siste forsøksdag.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som registreres om deg er fødselsdato, kjønn, samt informasjon fra ulike spørreskjema og undersøkelsene som blir utført. Det er kun prosjektleder og tilknyttede prosjektmedarbeidere som har tilgang til datamaterialet. Statens arbeidsmiljøinstitutt ved administrerende direktør er databehandlingsansvarlig. Vi ber også om samtykke til at du kan kontaktes for eventuell deltagelse i senere studier med lignende problemstillinger.

Utlevering av materiale og opplysninger til andre
Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger utleveres til samarbeidspartnere. Dette kan være land med lover som ikke tilfredsstiller europeisk personvernlovgivning.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi
Studien er finansiert gjennom interne forskningsmidler fra Statens arbeidsmiljøinstitutt og/eller ved midler fra Norges forskningsråd. Det er ingen interessekonflikter knyttet til studiens finansiering.

Forsikring
Deltakerne er dekket av en skadeforsikring tegnet for dette prosjektet.

Informasjon om utfallet av studien
Som deltaker i prosjektet har du rett til å informeres om resultatet i studien. Dette fås ved henvendelse til Dagfinn Matre.

Samtykke til deltakelse i studien

Jeg er villig til eventuelt å bli innbudt til en ekstra forsøksdag Ja / Nei

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

Appendix C

MATLAB script: Importing timestamps and VAS scores from raw data files to MATLAB

```
%% DEL 1
% Laster inn data

clear, close all
source_dir = '/Users/elinerodsjo/Documents/MATLAB/STAMI/cpm/cpm-korr';
files = dir(fullfile(source_dir, '*-cpm*.xlsx'));
SampleRate=512; %setter SampleRate

%% DEL 2
%Henter data fra excelfiler, klargjør for videre bruk

for i = 1:length(files)
    %henter subjektfil, i indikerer subjektnummer
    data = xlsread(fullfile(source_dir, files(i).name));

    % lager tabell med verdier for VASon og pain6 for alle subjekter
    VASon_pain6(i,1)=data(1,1); %legger subjektkode i kolonne 1

    % for loopen henter VASon og pain6 timestamp
    for ii=1:size(data,1) %ii indikerer radnummer
        if data(ii,5)==999 & data(ii,5)>data(ii+1,5) %finner VASon
           VASon_pain6(i,2)=data(ii+1,2); %legger VASon inn i tabellen

        elseif data(ii,4)==0 & data(ii+1,4)==1 %finner pain6
            VASon_pain6(i,3)=data(ii+1,2); %legger pain6 inn i tabellen
        end
    end

    %% DEL 3:
    % forberede VAS som er kompatibel med EEGdataen
    subject = data(1,1); %brukes til å navngi filer
    VAS_score = data(:,3); %kopierer VAS data fra excel
    VAS_upsampled = repelem(VAS_score,SampleRate);

    save(['VAS_upsampled_',num2str(subject)], 'VAS_upsampled')

end
save('VASon_pain6.mat','VASon_pain6');
```

Appendix D

MATLAB script: Adding timestamps and VAS scores to EEG files

```
%% DEL 1
%Laster inn tabell med tidsmarkører
%laster inn alle filer som benyttes i scriptet

clear, close all
cd('/Users/elinerodsjo/Documents/MATLAB/Preprosessering master EEG')
load VASon_pain6.mat
VASfiles = dir('*VAS_upsampled*.mat');
cd('/Users/elinerodsjo/Documents/MATLAB/STAMI/ExportEEG');
EEGfiles = dir('*cpm_512Hz*.mat'); %lister alle EEGfilene
length_experiment=120; %120 sekunder, 2 min
%dette scriptet kjøes før artefaktredigering av EEGfilene


%% DEL 2
%Legger inn VASscorer som en kanal
%legger inn tidsmarkører i hver enkelt EEG fil

for i = 1%:length(EEGfiles)
    cd('/Users/elinerodsjo/Documents/MATLAB/STAMI/ExportEEG');
    load(EEGfiles(i).name)  %laster inn et subjekt om gangen

    %lager markører for start- og stop-tid i eksperimentet
    ii = (VASon_pain6(i,3));
    %henter sekundverdi for subjekt(i) 2=VASon, 3=pain6
    exp_start = ii*SampleRate;
    % * sekundverdien med SampleRate fordi data er samplet til 512 Hz
    %"SampleRate" hentes fra EEGdatafilen
    exp_end = exp_start+(length_experiment*SampleRate);
    %finner slutttidspunkt for eksperimentet (VASon+lengde*samplerate)

    %legger til start-markør i EEGdata
    Markers(3).Position = exp_start;
    Markers(3).Type = 'time';
    Markers(3).Points = 1;
    Markers(3).Description = 'start';
    Markers(3).ChannelNumber = 0;

    %legger til slutt-markør i EEGdata
    Markers(4).Position = exp_end;
    Markers(4).Type = 'time';
    Markers(4).Points = 1;
    Markers(4).Description = 'end';
    Markers(4).ChannelNumber = 0;

    % lager VAS som "kanal" med samme lengde/sampling rate som EEGdataen
    load(VASfiles(i).name)
    %henter VAS_upsampled generert av forrige script (a)
    % fører til prompt "add its folder to the MATLAB path" --> svar ja
    VAS_x = zeros(size(VEOG));
    VAS_x(1:size(VAS_upsampled),1) = VAS_upsampled;
```

```
    %legger til info om 'VAS_upsampled' til variablene Channels & ChannelCount
    i EEGfilen
    Channels(34).Phi=0;
    Channels(34).Theta=0;
    Channels(34).Radius=0;
    Channels(34).Name='VAS_x';
    ChannelCount = 34;

    %lagrer endringer permanent i EEGdatafilen for hvert subjekt
    cd('/Users/elinerodsjo/Documents/MATLAB/Preprosessering master EEG')
    save(EEGfiles(i).name, 'VAS_x', 'Channels', 'ChannelCount', 'Markers','-
    append')

end
```

Appendix E

MATLAB script: Continuous wavelet transform, static EEG analysis

```
%dette scriptet tar i overkant av 1 time å kjøre
%% Overordnet kodestruktur

%nivå 1: Sette directory, åpne eeglab, definere konstante variabler, klargjøre
arrays
    % nivå 2, del 1: Batch processing loop (i) for å laste en EEGfil om gangen
    % sett inn info som skal stå utenfor ii-loop /klargjøre arrays osv.
       % nivå 3, del 1: Loop (ii) for analyse fra en kanal om gangen
           % steg 1.1 + 1.2: Definere signalet
           % steg 2: Definere nødvendig input info, gjøres også utenfor i-
loopen
           % steg 3: Kjøre continous wavelet analysis
           % steg 4: Produsere plots/output --> OPTIONAL
       % nivå 3, del 2, steg 5: lagre output i matrise
       % nivå 3, del 3: regne mean og sum for alle frekvensbånd
       % end nivå 3 (end ii-loop)
    % nivå 2, del 2, steg 5: Lagre nødvendige outputs i fil med tilhørende
subjektnummer
    % nivå 2, del 2: Slette minne av current fil før eeglab åpner ny fil
    % end nivå 2 (end i-loop)
 % lagre data fra alle subjekter i cross-subject-fil
 % end nivå 1, koden er ferdig

%%

%nivå 1
clear, close all;
cd('/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0');
eeglab;

source_dir =
'/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0/EEGLABfiler_filt50Hz';
%sjekk at du oppgir riktig mappe
EEGfiles_list = dir(fullfile(source_dir, '*clean1_filt50*.set')); %sjekk at
alle filnavn har en fellesnevner

%definere variabler
plot_spectral = 0; % endre til "= 1" om jeg ønsker å plotte spectral data for
alle kanalene per fil
channel_nr = 1:30; %antall kanaler, definerer antall rader i x,y,z matrisen +
antall runder ii-loopen kjører per fil
%1:30 heller enn 32 fordi knalene for VEOG og HEOG ikke skal v√¶re med i
analysen
nr_of_files = length(EEGfiles_list); %antall filer som skal analyseres (antall
unike eksperimentalsessions)
Fs = 512; %definerer samplerate, brukes av contwt (Fs = DT = tid mellom
datapunkter) og i utregning av EEG_total time
VAS_channel = 34; %definere hvilken kanal som inneholder VAS-score

%brukes i contwt
deltaJ=0.07;
```

```
%DEFAULT=0.25, brukes av contwt, setter intervallstørrelsen mellom scale-
faktorer, lavere verdi gir høyere oppløsning
s0_scale = 0.0078;
%DEFAULT=2*DT=(2*1/Fs), brukes av contwt, settter verdien av den minste scale-
faktoren (satt til ca 0.5 Hz, da dette er det laveste nivået jeg er
interesserte i å analysere)
J1_scale = 119;
%brukes av contwt, setter antallet scale-faktorer, altså hvor mange morlet-
waves av ulike størrelse som sammenlignes med signalet

%forbereder vektorer
VAS_mean = zeros(1,1);
%forberede array for VAS_mean
EEG_total_time = zeros(1,1);
%forberede array for EEG_total_time i sekunder
all_EEGfiles_total_time = zeros(nr_of_files,2);
%forberede array for å hente ut data om lengden av signalet (s) på tvers av
subjekter, hensikt: enklere tilgang senere
all_EEGfiles_mean_VAS = zeros(nr_of_files,2);
%forberede array for å hente ut data om mean VAS på tvers av subjekter,
hensikt: enklere tilgang senere
all_EEGfiles_frq_avg_power_pr_channel = zeros(channel_nr(end),5,nr_of_files);
%forberede array for å hente ut summert wavelet coefisienter på tvers av
subjekter, hensikt: enklere tilgang senere
check_files = zeros(length(EEGfiles_list),1);
%forberede vektor for å markere eventuelle filer med feil i tidsmarkør
frq_avg_power_pr_channel = zeros(channel_nr(end),6);
%forbereder subjekt-spesifikke matriser for plassering av sum av wavelet-coeff
per frekvensbpnd per kanal,  kolonner= 5 frekvensbånd(+ 1 kolonner for
kanalnummer), en verdi per frekvensbånd per kanal



%nivå 2, del 1
for i = 1:length(EEGfiles_list) %setter antall filer som skal analyseres
    [ALLEEG EEG CURRENTSET ALLCOM] = eeglab;
    EEG =
pop_loadset('filename',EEGfiles_list(i).name,'filepath','/Users/elinerodsjo/Do
cuments/MATLAB/eeglab2019_0/EEGLABfiler_filt50Hz'); %laster en fil, nummer "i"
    [ALLEEG, EEG, CURRENTSET] = eeg_store( ALLEEG, EEG, 0 );
    eeglab redraw;

    %forutsetning for steg 1, hensikt: sjekker at info om start og stop-
    tidspunkt i EEG dataen ligger på forventet plass,
    %if true --> henter ut tidspunkt for start&stop
    if all(EEG.event(2).description == 'start') & (EEG.event(end).description
    == 'end')
        start = EEG.event(2).latency;
        %finner starttidspunkt, hentes ut fra EEG.event.latency, rad 2
        stop  = EEG.event(end).latency;
        %finner stopptidspunkt, hentes ut fra EEG.event.latency, siste rad
    else %if not true, legger inn subjektfilnummer i check_files, jeg sjekker
    denne filen og kontrollerer evt filer som er markert
        check_files(i,1) = 1;
        continue
    end
```

```
VAS_score = EEG.data(VAS_channel,start:stop); %henter ut VAS-score for
eksperimentvinduet, start & stop er definert tidligere
VAS_mean = mean(VAS_score); %regner gjennomsnitt for VAS-score over
eksperimentvinduet

%definerer signalet (finne lengde av EEGdataen etter artefaktfjerning)
EEG_total_time (1,1) = length(EEG.data(:,start:stop))/Fs; %gir antall sec

%nivå 3, del 1
for ii = 1:channel_nr(end) %definert tidligere %ii=kanalnummer

    %steg 1
    Signal = (EEG.data(ii,start:stop)); %definerer signalet, altså hvilken
    kanal som analyseres i nåv√¶rende itterasjon av ii-loopen

    %steg 3
    [wave,period,scale,coi, dj, paramout, k] =
    contwt(Signal,1/Fs,[],deltaJ,s0_scale,J1_scale);
    % utfører cwt, benytter konstanter som er definert tidligere


    if ii == 1
        x = length(Signal);
        % representerer tid/length of signal
        y = length(scale);
        % representerer scales -  konverteres til pseudofrekvens senere
        z = channel_nr(end);
        % er definert tidligere, antall kanaler
        spectral_data = zeros(x,y,z);

        %konvertere scales til pseudo-frekvens
        pf = scal2frq(scale,"morl");
        %følgende 2 linjer trengs ikke, med mindre man vil eksportere
        %tabellen med oversikt over hvilke pseudofrekvenser som
        %tilsvarer hvilke scales
        %T = [scale(:) pf(:)];
        %T = array2table(T,'VariableNames',{'Scale','Pseudo_Frequency'});

        %finne grenseverier for frekvensbåndene, til bruk for å indeksere
        i spectral_data
        %delt inn i 5 frekvensbånd, lagt til rette for subindeling av
        enkelte bånd dersom rom for utvidelse av analyse

        find_delta = find(pf>=1 & pf<=4); %delta (1,Äì4 Hz)
        find_theta = find(pf>=4 & pf<=8 ); %theta (4,Äì8 Hz)
        find_alpha = find(pf>=8 & pf<=12 ); %alpha (8,Äì12 Hz)
        %find_alpha1 = find(pf>=8 & pf<=10 ); %alpha1 (8,Äì10 Hz)
        %find_alpha2 = find(pf>=10 & pf<=12 ); %alpha2 (10,Äì12 Hz)
        find_beta = find(pf>=12 & pf<=32 ); %beta (12,Äì32 Hz)
        %find_beta1 = find(pf>=12 & pf<=18 ); %beta1 (12,Äì18 Hz)
        %find_beta2 = find(pf>=18 & pf<=24 ); %beta2 (18,Äì24 Hz)
        %find_beta3 = find(pf>=24 & pf<=32 ); %beta3 (24,Äì32 Hz)
        find_gamma = find(pf>=32 & pf<=70 ); %gamma (32,Äì70 Hz)
    end
```

```
%steg 4
if plot_spectral == 1 %plotte data
    figure(ii); clf;
    subplot(211)
    plot(Signal)
    subplot(212)
    imagesc(abs(wave));
    %dersom du ønsker plot, bytte ut "wave" med å indexe inn i et
    frekvensbånd i spectral_data
end

%nivå 3, del 2, steg 5
spectral_data(:,:,ii) = wave';
%plasserer wavelet-coeff inn i cross-channel variabel

%regne sum (av gjenomsnitt av absoluttveridene i wave per scale-
faktor) for hvert frekvensbånd innad i en kanal  + plassere i subjekt-
spesifikk tabell
%finner først gjenomsnittet over hele signalet innen hver scale per
Kanal, summerer derreter alle scales innen et frekvensbånd per kanal

%dyn_spectral_data(:,find_delta,p,u) - definerer område i matrisen som
har relevant data for et gitt frekvensbånd
%hvor (:,find_XX,ii) = hele lengden av signalet, alle scale-faktorer
som tilsvarer et gitt frekvensbånd, innad kanal ii
%abs(dyn_spectral_data) fordi vi ønsker absoluttvedien
%mean(abs(dyn_spectral_data)) fordi vi ønsker et gjenomsnittet for
hele signalet per scale-faktor, alså per kolonne i matrisen
%sum(mean(abs(dyn_spectral_data(z,x,z,w))), 'all') fordi vi ønsker en
samlet sum for gjenomsnittene i det definerte dataområdet

frq_avg_power_pr_channel(ii,1)=ii; %kanalnummer
frq_avg_power_pr_channel(ii,2) =
sum(mean(abs(spectral_data(:,find_delta,ii))),'all');
%aktivitet innen deltabåndet,
frq_avg_power_pr_channel(ii,3) =
sum(mean(abs(spectral_data(:,find_theta,ii))),'all');
frq_avg_power_pr_channel(ii,4) =
sum(mean(abs(spectral_data(:,find_alpha,ii))),'all');
%frq_avg_power_pr_channel(ii,4) =
sum(mean(abs(spectral_data(:,find_alpha1,p))),'all');
%frq_avg_power_pr_channel(ii,5) =
sum(mean(abs(spectral_data(:,find_alpha2,p))),'all');
frq_avg_power_pr_channel(ii,5) =
sum(mean(abs(spectral_data(:,find_beta,ii))),'all');
%frq_avg_power_pr_channel(ii,6) =
sum(mean(abs(spectral_data(:,find_beta1,p))),'all');
%frq_avg_power_pr_channel(ii,7) =
sum(mean(abs(spectral_data(:,find_beta2,p))),'all');
%frq_avg_power_pr_channel(ii,8) =
sum(mean(abs(spectral_data(:,find_beta3,p))),'all');
frq_avg_power_pr_channel(ii,6) =
sum(mean(abs(spectral_data(:,find_gamma,ii))),'all');

%T_frq_avg_power_pr_channel = array2table(frq_avg_power_pr_channel,
'VariableNames', {'Channel_number','delta','theta','alpha1','alpha2',
```

```
        'beta1', 'beta2', 'beta3', 'gamma'});
        T_frq_avg_power_pr_channel = array2table(frq_avg_power_pr_channel,
        'VariableNames',{'Channel_number','delta', 'theta', 'alpha',
        'beta', 'gamma'});


    end

    %legge data i cross-subject filer med subjektkoder for EEG_total_time &
    VAS_mean, og fil med sum for frekvensbånd(kanalvis) for alle deltagere

    all_EEGfiles_total_time(i,1) = str2double(EEGfiles_list(i).name(2:4));
    all_EEGfiles_total_time(i,2) = EEG_total_time;
    T_all_EEGfiles_total_time = array2table(all_EEGfiles_total_time,
    'VariableNames', {'Subject_number','Total_time_sec'});

    all_EEGfiles_mean_VAS(i,1) = str2double(EEGfiles_list(i).name(2:4));
    all_EEGfiles_mean_VAS(i,2) = VAS_mean;
    T_all_EEGfiles_mean_VAS = array2table(all_EEGfiles_mean_VAS,
    'VariableNames',{'Subject_number','VAS_mean'});

    all_EEGfiles_frq_avg_power_pr_channel(:,:,i) =
    (frq_avg_power_pr_channel(:,2:6));
    %trenger ikke ta med konlonne for å skrive kanalnummer i 3Dmatrisen

    %kan evt sette inn kode fra script_save_for_export her (+lenger oppe)

    %nivå 2, del 2, steg 5
    %save([(EEGfiles_list(i).name(1:end-4)),'_spectral'],'spectral_data',
    'VAS_score', 'VAS_mean', 'EEG_total_time',
    %spectral_data overstiger 2GB og tar lang tid å lagre
    /ikke mulig uten MATfile version 7.3

    %lagre en fil uten 'spectral data' så det går fortere å laste inn ved
    senere bruk
    save([(EEGfiles_list(i).name(1:end-4)),'_frq_sum_stat'],'VAS_score',
    'VAS_mean', 'EEG_total_time', 'frq_avg_power_pr_channel',
    'T_frq_avg_power_pr_channel');

    %nivå 2, del 2
    EEG = pop_delset( EEG, [1] ); %fjerne minnet om feridg prosessert EEG-fil
    før ny lastes inn

end

%nivå 1

filename = 'all_EEGfiles_stat';
save(filename, 'T_all_EEGfiles_total_time', 'T_all_EEGfiles_mean_VAS',
'all_EEGfiles_frq_avg_power_pr_channel');
%dersom denne koden kjøres puljevis, må "all_EEG_files_stat"-filene lagres med
ulike navn manuelt for at de ikke skal skrives over av neste runde
```

```
%% SAMLING AV DATA + EKSPORT AV DATA FRA .MAT TIL EXCEL
%denne delen av scriptet tar <1 min å kjøre
%sjekk at filer generert av scriptet over er flyttet til riktig mappe
%Sjekk at antall filer i mappen EEGLABfiler_frq_avg(.mat) er som foventet
(n=92)

%oppgavene i dette scripet kan enten gjøres med en egen for-løkke som
%henter inn hver enkelt subjektfil ELLER lagre underveis i
spectralanalyseloopen (scriptet over)
%jeg velger egen for-løkke for at det skal v√¶re enklere å kjøre koden/fikse
feil i koden når den er delt inn i funksjonelle moduler

clear, close all;

cd('/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0');
load marker_sleep_condition.mat %laster info om søvnbetingelse og ID-markør/K-
code

cd('/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0/EEGLABfiler_frq_sum(.mat)
'); %path til mappen med filer som skal brukes
source_dir =
'/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0/EEGLABfiler_frq_sum(.mat)';
EEGfiles_list = dir(fullfile(source_dir, '*_frq_sum_stat*.mat')); %velger
hvilke filer som skal lastes inn basert på fellesnevner i filnavn

data = zeros(length(EEGfiles_list),12); %forberede matrise
%lage header-rad
%header =
{'ID','code','sleep_condition','VAS_mean','delta','theta','alfa1','alfa2','bet
a1','beta2','beta3','gamma','KSS','MRT'};
header =
{'ID','code','sleep_condition','VAS_mean','delta','theta','alfa','beta','gamma
','KSS','MRT','time'};

%legge inn data i matrix

for i=1:length(EEGfiles_list)
    load(EEGfiles_list(i).name) %laster inn fil


    subject_code = str2double(EEGfiles_list(i).name(2:4));
    %finner subjektkoden, med utgangspunkti filnavn
    if subject_code==264
    %fjerner data fra noen kanaler fordi par-fil mangler data
        p = [10 12 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30];
    elseif subject_code==264
    %fjerner data fra noen kanaler fordi par-fil mangler data
        p = [15 16 17 18 19 20 22 23 24 25 26 27 28 29 30];
    else
        p = 1:30;
    end

    %regner ut sum power i frekvensbånd på tvers av kanaler (med utgangspunkt
    i forrige script, hvor jeg regnet  gjenomsnitt av wavelet koeffisientene
    over tid innad i hver scale-faktor, og deretter summerte gjenomsnittene
```

```
innad i hvert frekvensbånd per kanal

sum_delta = sum(frq_avg_power_pr_channel(p,2));
%summerer tall-verdier fra alle kanalene innad i delta-båndet
sum_theta = sum(frq_avg_power_pr_channel(p,3));
sum_alpha = sum(frq_avg_power_pr_channel(p,4));
%mean_alfa1 = mean(frq_avg_power_pr_channel(p,4));
%mean_alfa2 = mean(frq_avg_power_pr_channel(p,5));
%alternavtiv dersom subindeling av frekvensbånd er inkludert i analysen
sum_beta = sum(frq_avg_power_pr_channel(p,5));
%mean_beta1 = mean(frq_avg_power_pr_channel(p,6));
%mean_beta2 = mean(frq_avg_power_pr_channel(p,7));
%mean_beta3 = mean(frq_avg_power_pr_channel(p,8));
%alternavtiv dersom subindeling av frekvensbånd er inkludert i analysen
sum_gamma = sum(frq_avg_power_pr_channel(p,6));


%huskeliste for plassering av data (12 kolonner)
    %data(:,1) = ID
    %data(:,2) = code
    %data(:,3) = markør for søvnbetingelse
    %data(:,4) = VAS_mean (mean VAS)
    %data(:,5) = d (delta)
    %data(:,6) = t (theta)
    %data(:,7) = a (alpha)
    %data(:,8) = b (beta)
    %data(:,9) = g (gamma)
    %data(:,10) = KSS (Karolinska Sleepiness scale),
    limes inn i excel senere
    %data(:,11) =  mean reaction time,
    limes inn i excel senere
    %data(:,12) =  lengden av signalet (s) etter artefakt fjerning

%henter info om ID og søvnbetingelse fra "marker_sleep_condition.mat"
K = find(sleep_condition_marker(:,2)==subject_code);

%plasserer data
data(i,1) = sleep_condition_marker(K,1);
data(i,2) = str2double(EEGfiles_list(i).name(2:4));
data(i,3) = sleep_condition_marker(K,3);
data(i,4) = VAS_mean;
data(i,5) = sum_delta;
data(i,6) = sum_theta;
data(i,7) = sum_alpha;
%data(i,X) = sum_alfa1;
%data(i,X) = sum_alfa2;
data(i,8) = sum_beta;
%data(i,X) = sum_beta1;
%data(i,X) = sum_beta2;
%data(i,X) = sum_beta3;
data(i,9) = sum_gamma;
data(i,12) = EEG_total_time(1,1);

%sjekk at alle subjekt-spesifikke variabler fjernes før neste iterasjon av
loopen.
%IKKE clear all, vi trenger at 'data' forblir i workspace
```

```
    clear VAS_mean frq_avg_power_pr_channel EEG_total_time
    T_frq_avg_power_pr_channel

end

%konkatenere header og datamatrix
spectral_analysis = [header;num2cell(data)];

%lagre som .mat fil
save('all_EEGfiles_spectral_analysis', 'spectral_analysis');

%lagre som excel fil

xls_spectral_analysis =array2table(num2cell(data),
'VariableNames',{'ID','code','sleep_condition','VAS_mean','delta','theta','alf
a','beta','gamma','KSS','MRT','time'});
%alternavtiv dersom subindeling av frekvensbånd er inkludert i analysen
%xls_spectral_analysis =array2table(num2cell(data),
'VariableNames',{'ID','code','sleep_condition','VAS_mean','delta','theta','alf
a1','alfa2','beta1','beta2','beta3','gamma','KSS','MRT'});

T=xls_spectral_analysis;
filename = 'all_EEGfiles_spectral_analysis_1603.xlsx';
writetable(T,filename,'Sheet',1,'Range','A1')
```

Appendix F

MATLAB script: Continuous wavelet transform, dynamic EEG analysis

```
%% dynamic spectral analysis (både contwt & moving average VAS)

clear, close all;
cd('/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0');
eeglab; %åpner eeglab

load marker_sleep_condition.mat
%laster info om søvnbetingelse og ID-markør/K-code

source_dir =
'/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0/EEGLABfiler_filt50Hz';
%path til mappen med filer som skal benyttes i analysen
EEGfiles_list = dir(fullfile(source_dir, '*clean1_filt*.set'));
%velger hvilke filer som skal lastes inn basert på fellesnevner i filnavn

%definere variabler og forberede vektorer
VAS_channel = 34;
%definerer hvilken kanal som inneholde VAS-score
EEG_chan = 1:30;
%definerer hvilke kanaler som inneholder relevant EEGdata (her er HEOG og VEOG
kanalene fjernet)
check_files = zeros(length(EEGfiles_list), 1);
%forberede vektor for å markere eventuelle filer med feil i tidsmarkør
time_length = zeros(1,length(EEGfiles_list));
%forberede vektor for å lagre info om hvor mange 15 sek tidsperioder hvert
signal har

Fs = 512;
%definerer samplerate, brukes av contwt (Fs = DT = tid mellom datapunkter)
%og i utregning av EEG_total time
win_sec = 15;
%antall sekund for vindustørrelse som skal brukes i analysen
win_size = Fs*win_sec;
%antall kolonner i signalmatrisen som tilsvarer 15 sek %samplerate = 512,
%hvert 512te kolonne = ett sekund 15 sek = 512x15 = 7680
step_size = win_size/2;
%gir 50% overlapp mellom hvert vindu, i dette tilfellet 7.5 sek overlapp

%definere konstaner, brukes i contwt
deltaJ=0.07; %DEFAULT=0.25
%brukes av contwt, setter intervallstørrelsen mellom scale-faktorer,
%lavere verdi gir høyere oppløsning
s0_scale = 0.0078; %DEFAULT=2*DT=(2*1/Fs)
%brukes av contwt, settter verdien av den minste scale-faktoren
%(satt til ca 0.5 Hz, da dette er det laveste nivået jeg er interesserte i)
J1_scale = 119;
%brukes av contwt, setter antallet scale-faktorer
%altså hvor mange morlet-waves av ulike størrelse som sammenlignes med
signalet

%nivå 00, del 1
for i = 1:length(EEGfiles_list)
```

```
%setter antall filer i-loopen behandler = antall "obervasjoner" i studien
[ALLEEG EEG CURRENTSET ALLCOM] = eeglab;
EEG = pop_loadset('filename',EEGfiles_list(i).name,
'filepath','/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0/
EEGLABfiler_filt50Hz');
%laster inn subjekt-fil
[ALLEEG, EEG, CURRENTSET] = eeg_store( ALLEEG, EEG, 0 );
eeglab redraw;

%sjekker at info om start og stoptidspunkt ligger på forventet plass,
%if true --> henter ut tidspunkt for start&stop
if all(EEG.event(2).description == 'start') & (EEG.event(end).description
== 'end')
    start = EEG.event(2).latency;
    %finner starttidspunkt, hentes ut fra EEG.event.latency, rad 2
    stop  = EEG.event(end).latency;
    %finner stopptidspunkt, hentes ut fra EEG.event.latency, siste rad
else %if not true, legger inn subjektfilnummer i check_files
    check_files(i,1) = str2double(EEGfiles_list(i).name(2:4));
    continue
end

EEG_signal = EEG.data(EEG_chan(1:end),start:stop);
%lager matrise med relevant EEGdata, med tidligere definerte EEG-kanaler
og tidsmarkører
VAS_signal = EEG.data(VAS_channel,start:stop);
%definerer VAS-signalet,med tidligere definerte VAS-kanal og tidsmarkører


step_values = 1:step_size:length(VAS_signal)-win_size;
%finner verdien for begynnelsen av alle tidsvindu som skal analyseres,
brukes av for-løkka (i), %-win_size for å unngå erroren "exceeded array
bounds"

dyn_VAS_mean = zeros(1,length(step_values));
%forbereder vektor for å lagre mean VAS-score for hvert tidsvindu

subject_code = str2double(EEGfiles_list(i).name(2:4));
%finner subjektkode med utgangspunkt i fil-navn
K = find(sleep_condition_marker(:,2)==subject_code);
%finner indx for plassering av subjektkoden i "marker_sleep_condition.mat"
dyn_2D_spectral_data = zeros(length(step_values),10);
%forbereder matrise for å holde data om hvert frekvensbånd + id,code ++,
antall rader=antall tidsvindu
time_length(1,i) = length(step_values);
%plasserer info om hvor mange 15 sek tidsperioder hvert signal/subjekt har

    for v = 1:step_size:length(VAS_signal)-win_size
    % evt step_values(1:end)
        indx = find(step_values==v);
        %finner iterasjonnummer for loopen ved å matche verdien av ii til
        tilsvarende verdi i step_values
        dyn_VAS_mean(1,indx) = mean(VAS_signal(1,v:v+win_size));
        %plasserer meanVAS for et gitt tidsvindu (v) i en subject—
        spesifikk vektor
    end
```

```
for ii = 1:EEG_chan(end)
% for-løkke for å kunne analysere en kanal om gangen

        for iii = 1:step_size:length(EEG_signal)-win_size
            Signal_dyn = EEG_signal(ii,iii:iii+win_size-1);
            %ii = kanalnr, iii:iii+win_size-1 = current start av vindu
            til start+vindusstørrelse -1
            [wave,period,scale,coi, dj, paramout, k] =
            contwt(Signal_dyn,1/Fs,[],deltaJ,s0_scale,J1_scale);
            %utfører CWT

            indx = find(step_values==iii);
            %finner iterasjonnummer for loopen ved å matche verdien av
            ii til tilsvarende verdi i step_values

            pf = scal2frq(scale,"morl");
            %oversetter scale-verdi til pseudo-frekvens
            find_delta = find(pf>=1 & pf<=4); %delta (1,Äì4 Hz)
            find_theta = find(pf>=4 & pf<=8 ); %theta (4,Äì8 Hz)
            find_alpha = find(pf>=8 & pf<=12 ); %alpha (8,Äì12 Hz)
            find_beta = find(pf>=12 & pf<=32 ); %beta (12,Äì32 Hz)
            find_gamma = find(pf>=32 & pf<=70 ); %gamma (32,Äì70 Hz)

            %forbereder matrise for spektraldataen under første
            itterasjon av for-løkka

             if iii==1
                x = win_size; %(length(Signal_eeg));
                % representerer tid, lengden av signalet som
                analyseres
                y = length(scale); % representerer pseudo-frekvens
                z = EEG_chan(end); % representerer de ulike kanalene,
                er definert tidligere
                w = length(step_values); %er lik antall tidsvindu i i-
                loopen
                dyn_spectral_data = zeros(x,y,z,w);
                %altså er data (:,:,:,1) alle koeffisientene i
                tidsvindu 1, (:,:,:,2) = tidsvindu 2
             end

             dyn_spectral_data(:,:,ii,indx) = wave';
             %evt abs(wave) %plasserer koeffisientene fra output
             "wave" i subject-spesifikk 4D-matrise

        end

end

for u=1:length(step_values)
%for-løkke plasserer data fra hvert frekvensbånd innad i hvert
tidsvindu i en 2D-matrise

    if subject_code==264
    %fjerner data fra noen kanaler fordi par-fil mangler data
```

```
            p = [10 12 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
            30];
        elseif subject_code==264
        %fjerner data fra noen kanaler fordi par-fil mangler data
            p = [15 16 17 18 19 20 22 23 24 25 26 27 28 29 30];
        else
            p = EEG_chan; %1:30
        end

        dyn_2D_spectral_data(:,1) = sleep_condition_marker(K,1);
        %legger inn ID-nummer, fra filen marker_sleep_condition
        dyn_2D_spectral_data(u,2) = subject_code;
        % legger inn K-nummer
        dyn_2D_spectral_data(:,3) = sleep_condition_marker(K,3);
        %legger inn søvnbetingelse
        dyn_2D_spectral_data(u,4) = u;
        % markerer rekkefølgen på tidsvindu (1:n)
        dyn_2D_spectral_data(u,5) = dyn_VAS_mean(1,u);
        %legger inn mean VAS for hvert tidsvindu

        %finner gjenomsnitt av koeffisientene for signalet innad i hver
        scale-faktor,
        %summerer deretter alle gjenomsnitt-koeffisientene innad i hvert
        frekvensbånd per kanal
            %dyn_spectral_data(:,find_delta,p,u) - definerer område i
            matrisen som har relevant data for et gitt frekvensbånd
            %hvor (:,find_XX,p,u) = lengden av signalet (15 sek), alle
            scale-faktorer som tilsvarer et gitt frekvensbånd, alle
            kanaler i tidsvindu "u"
            %abs(dyn_spectral_data) fordi vi ønsker absoluttvedien
            %mean(abs(dyn_spectral_data)) fordi vi ønsker et gjenomsnittet
            for det definerte signalet per scale-faktor, alså per rad i
            matrisen
            %sum(mean(abs(dyn_spectral_data(z,x,z,w))), 'all') fordi vi
            ønsker en samlet sum for gjenomsnittene i det definerte
            dataområdet

        dyn_2D_spectral_data(u,6) = sum(mean(abs(dyn_spectral_data
        (:,find_delta,p,u))),'all');
        dyn_2D_spectral_data(u,7) = sum(mean(abs(dyn_spectral_data
        (:,find_theta,p,u))),'all');
        dyn_2D_spectral_data(u,8) = sum(mean(abs(dyn_spectral_data
        (:,find_alpha,p,u))),'all');
        dyn_2D_spectral_data(u,9) = sum(mean(abs(dyn_spectral_data
        (:,find_beta,p,u))),'all');
        dyn_2D_spectral_data(u,10) = sum(mean(abs(dyn_spectral_data
        (:,find_gamma,p,u))),'all');
    end

save([(EEGfiles_list(i).name(1:end-4)),'_dyn_spectral_sum'],
'dyn_2D_spectral_data', 'dyn_VAS_mean');

%evt %save([(EEGfiles_list(i).name(1:end-4)),'_dyn_spectral'],
'dyn_spectral_data','dyn_VAS_mean');
% men 'dyn_spectral_data' gjør filen for stor til p lagres effektivt
```

```
    EEG = pop_delset( EEG, [1] );
    %fjerne minnet om feridg prosessert EEG-fil før ny lastes inn

end


filename = 'all_EEGfiles_dyn_stat'
save(filename, 'time_length');

%% konkatenerer alle subjekt-spesifikke filer til en cross-subject fil

clear close all

load all_EEGfiles_dyn_stat.mat
source_dir = '/Users/elinerodsjo/Documents/MATLAB/eeglab2019_0';
%sjekk at riktig mappe er oppgitt
EEGfiles_list = dir(fullfile(source_dir, '*_dyn_spectral_sum*.mat'));
%sjekk at alle filnavn har en fellesnavner

dyn_data_long_format = zeros(sum(time_length),10);

%formatere data til lengdeformat, for statistisk testing
for i=1:length(EEGfiles_list)
    load(EEGfiles_list(i).name)
    %laster inn alle subjkt-spesifikke filer, en om gangen
    indx = find(dyn_data_long_format(:,1)==0);
    %finner første tomme rad, legger ny "tabell" inn under forrige
    dyn_data_long_format((indx(1):indx(1)+time_length(i)-1),:) =
    dyn_2D_spectral_data(:,:);
    clear dyn_2D_spectral_data dyn_VAS_mean
end

dyn_data_wide_format = zeros(length(EEGfiles_list)*6,max(time_length)+3);

%breddeformat, for plotting
%
% for i=1:length(EEGfiles_list)
%     load(EEGfiles_list(i).name)
%     %laster inn alle subjkt-spesifikke filer, en om gangen
%     at = size(dyn_2D_spectral_data,1);
%
%     dyn_data_wide_format(i*6-5:i*6,1) = dyn_2D_spectral_data(1,1); %ID
%     dyn_data_wide_format(i*6-5:i*6,2) = dyn_2D_spectral_data(1,2); %code
%     dyn_data_wide_format(i*6-5:i*6,3) = dyn_2D_spectral_data(1,3); %sleep
%     dyn_data_wide_format(i*6-5,4:3+at) = dyn_2D_spectral_data(:,5)'; %VAS
%     dyn_data_wide_format(i*6-4,4:3+at) = dyn_2D_spectral_data(:,6)'; %delta
%     dyn_data_wide_format(i*6-3,4:3+at) = dyn_2D_spectral_data(:,7)'; %theta
%     dyn_data_wide_format(i*6-2,4:3+at) = dyn_2D_spectral_data(:,8)'; %alfa
%     dyn_data_wide_format(i*6-1,4:3+at) = dyn_2D_spectral_data(:,9)'; % beta
%     dyn_data_wide_format(i*6-0,4:3+at) = dyn_2D_spectral_data(:,10)'; %gamma
%
%     clear dyn_2D_spectral_data dyn_VAS_mean
%
% end
```

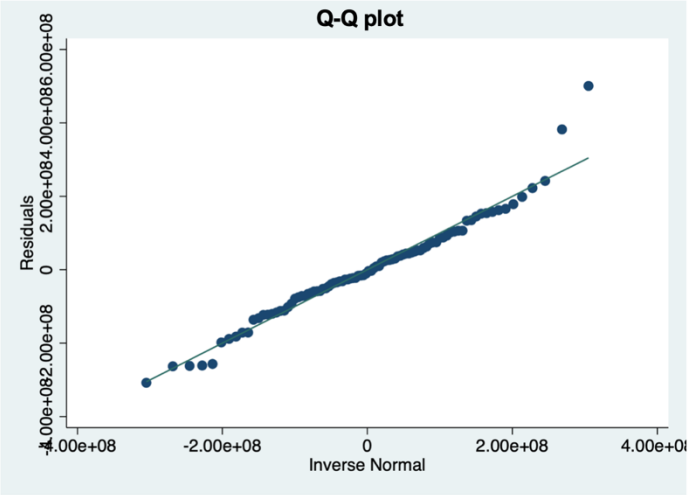
```
delta_plot=zeros(length(EEGfiles_list),max(time_length)+1);
theta_plot=zeros(length(EEGfiles_list),max(time_length)+1);
alpha_plot=zeros(length(EEGfiles_list),max(time_length)+1);
beta_plot=zeros(length(EEGfiles_list),max(time_length)+1);
gamma_plot=zeros(length(EEGfiles_list),max(time_length)+1);
VAS_plot=zeros(length(EEGfiles_list),max(time_length)+1);

for i=1:length(EEGfiles_list)
    load(EEGfiles_list(i).name)
    %laster inn alle subjkt-spesifikke filer, en om gangen
    at = size(dyn_2D_spectral_data,1);


    delta_plot(i,1) = dyn_2D_spectral_data(1,3); %sleep
    delta_plot(i,2:1+at) = dyn_2D_spectral_data(:,6)'; %delta
    theta_plot(i,1) = dyn_2D_spectral_data(1,3); %sleep
    theta_plot(i,2:1+at) = dyn_2D_spectral_data(:,7)'; %theta
    alpha_plot(i,1) = dyn_2D_spectral_data(1,3); %sleep
    alpha_plot(i,2:1+at) = dyn_2D_spectral_data(:,8)'; %alfa
    beta_plot(i,1) = dyn_2D_spectral_data(1,3); %sleep
    beta_plot(i,2:1+at) = dyn_2D_spectral_data(:,9)'; % beta
    gamma_plot(i,1) = dyn_2D_spectral_data(1,3); %sleep
    gamma_plot(i,2:1+at) = dyn_2D_spectral_data(:,10)'; % gamma
    VAS_plot(i,1) = dyn_2D_spectral_data(1,3); %sleep
    VAS_plot(i,2:1+at) = dyn_2D_spectral_data(:,5)'; %VAS

    clear dyn_2D_spectral_data dyn_VAS_mean
end

filename = 'all_EEGfiles_dyn_stat';
save(filename, 'time_length', 'dyn_data_long_format', 'dyn_data_wide_format',
'delta_plot','theta_plot', 'alpha_plot', 'beta_plot', 'gamma_plot', 'VAS_plot'
);

xls_dyn_data_long_format =array2table(num2cell(dyn_data_long_format),
'VariableNames',{'ID','code','sleep_condition','time','VAS_mean','delta','thet
a','alfa','beta','gamma'});
T=xls_dyn_data_long_format;
filename = 'all_EEGfiles_dyn_spectral_analysis.xlsx';
writetable(T,filename,'Sheet',1,'Range','A1')
```

Appendix G

Example of Q-Q plots

Figure G1

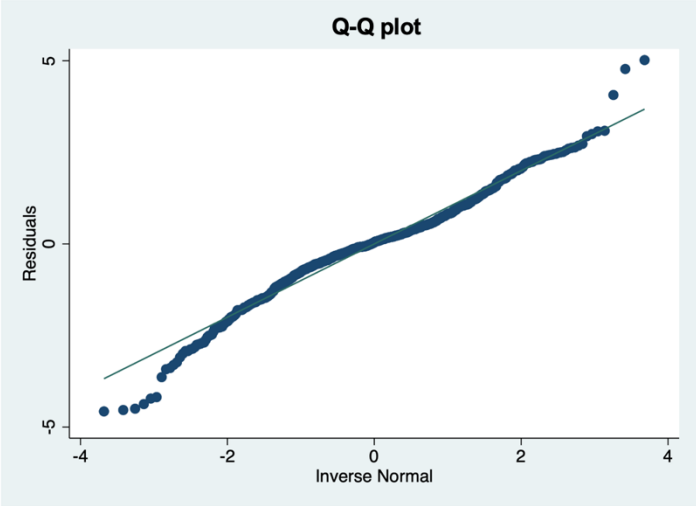
Q-Q plot of residuals from the static delta band model



Note. Overall, the line looks straight, suggesting that the assumption of normal distribution of residuals is not violated. The Q-Q plot indicates that there are a couple of outliers. These could be investigated further and considered removed from the dataset.

Figure G2

Q-Q plot of residuals from the dynamic delta band model



Note. Overall, the line looks straight, suggesting that the assumption of normal distribution of residuals is not violated. However, there are some deviations from the expected line towards the tails. To improve the relative normality of the data further, one could consider transforming the dataset.

Appendix H

Liner Mixed Model summaries of static EEG analyses

Table H1

Linear mixed model summary of variables predicting Delta EEG activity

Fixed effects	Coefficient (absolute activity in au)	95% CI	*p*-value
Sleep	1.64	[-4.94, 8.22]	.626
Cons	101.00	[95.50, 107.00]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. Coefficients and CIs in this table are divided by e+07.

Table H2

Linear mixed model summary of variables predicting Theta EEG activity

Fixed effects	Coefficient (absolute activity in au)	95% CI	*p*-value
Sleep	0.81	[-1.05, 2.67]	.394
Cons	29.6	[0.27, 0.32]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. Coefficients and CIs in this table are divided by e+07.

Table H3

Linear mixed analysis summary of variables predicting Alpha EEG activity

Fixed effects	Coefficient (absolute activity in au)	95% CI	*p*-value
Sleep	0.09	[-0.87, 1.06]	.850
Cons	13.00	[12.00, 15.00]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. Coefficients and CIs in this table are divided by e+07.

Table H4

Linear mixed model summary of variables predicting Beta EEG activity

Fixed effects	Coefficient (absolute activity in au)	95% CI	*p*-value
Sleep	0.36	[-1.31, 2.03]	.671
Cons	22.60	[20.8, 24.40]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. Coefficients and CIs in this table are divided by e+07.

Table H5

Linear mixed model summary of variables predicting Gamma EEG activity

Fixed effects	Coefficient (absolute activity in au)	95% CI	*p*-value
Sleep	0.13	[-1.26, 1.52]	.858
Cons	13.40	[12.30, 14.50]	<.001

Note. Reference group for the variable sleep condition is habitual sleep. Coefficients and CIs in this table are divided by e+07.

Appendix I

Liner Mixed Model summaries of dynamic EEG analyses

Table I1

Linear mixed model summary of variables predicting dynamic VAS-scores (delta band)

Fixed effects	Coefficient (VAS in cm)	95% CI	*p*-value
Sleep	1.519	[0.622, 2.414]	.001
Time	0.063	[0.031, 0.094]	<.001
Delta	-0.001	[-0.001, 0.001]	.620
Sleep#Delta	-0.001	[-0.002, 0.001]	.320
Cons	4.622	[3.716, 5.528]	<.001

Note. Reference group for the variable sleep condition is habitual sleep.

Table I2

Linear mixed model summary of variables predicting dynamic VAS-scores (beta band)

Fixed effects	Coefficient (VAS in cm)	95% CI	*p*-value
Sleep	0.774	[0.104, 1.444]	.024
Time	0.066	[0.034, 0.098]	<.001
Beta	-0.001	[-0.003, 0.002]	.529
Sleep#Beta	0.001	[-0.002, 0.005]	.426
Cons	4.619	[3.756, 5.482]	<.001

Note. Reference group for the variable sleep condition is habitual sleep.

Table I3

Linear mixed model summary of variables predicting dynamic VAS-scores (gamma band)

Fixed effects	Coefficient (VAS in cm)	95% CI	*p*-value
Sleep	0.598	[0.065, 1.130]	.028
Time	0.067	[0.035, 0.098]	<.001
Gamma	-0.002	[-0.006, 0.002]	.248
Sleep#Gamma	0.004	[-0.001, 0.008]	.086
Cons	4.699	[3.883, 5.514]	<.001

Note. Reference group for the variable sleep condition is habitual sleep.