Shift work and pain: A quantitative EEG investigation into the effects of shift work and nocebo on pain perception.

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Abstract

Introduction: Shift work is associated with increased prevalence of pain and shift workers commonly report reduced sleep, which is related to increased pain sensitivity. Thus, night shift work (NSW) may potentially lead to increased pain sensitivity. This study investigates electroencephalogram (EEG) recordings in response to nociceptive electrical stimuli following NSW and habitual sleep (HS) with and without negative expectation (nocebo).

Methods: 53 nurses participated in the study. They received nociceptive electrical stimuli following NSW and HS that were either correctly signalled or signalled as higher than the actual intensity delivered (in the case of nocebo). Pain scores were recorded using a visual analogue scale (1-10). EEG measurements were recorded from 32 electrodes and analysed in the time-frequency domain using Analyzer, EEGlab and Matlab. Linear Mixed Models in SPSS was used for statistical analysis.

Results: Following NSW, the participants exhibited increased event-related synchronisation (ERS) in response to nociceptive stimuli in the 1-400 ms/1-25 Hz, post stimulus interval across several electrodes, which was significant at $p < 0.05$ level. Nocebo was significantly associated with lower ERS magnitude than correctly signalled stimuli ($p < 0.05$). Finally, there was a significant effect of NSW and nocebo on pain scores ($p < 0.05$), in which the participants rated the electrical stimuli as more painful following NSW and nocebo, however, nocebo was not facilitated by NSW ($p = 0.438$).

Conclusion: NSW leads to sleep induced hyperalgesia accompanied by increased ERS across several electrodes following exposure to nociceptive electrical stimuli. There is also hyperalgesia in response to nocebo, which is accompanied by reduced ERS compared to correctly signalled stimuli. However, the present study does not find support for nocebo as a principal underlying factor in SIH, but rather, SIH and NIH appear to stem from cortical processes that do not overlap.

Preface

This master's thesis is part of an ongoing research project at the National Institute of Occupational Health (STAMI) investigating the effects of shift work on pain perception. The study group is led by:

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Contents

Abbreviations

ACC: anterior cingulate cortex

α-ERD: event-related desynchronisation in the alpha frequency bandwidth

EEG: electroencephalogram

EMM: estimated marginal means

ERD: event-related desynchronisation

ERP: event-related potential

ERS: event-related synchronisation

GBOs: gamma band oscillations

HS: habitual sleep

LMM: linear mixed model

MEG: magnetoencephalogram

NIH: nocebo-induced hyperalgesia

NSW: night shift work

PCC: posterior cingulate cortex

PFC: pre-frontal cortex

REM: rapid eye movement

ROI: region of interest

SI: primary somatosensory cortex

SII: secondary somatosensory cortex

SIH: sleep-induced hyperalgesia

SR: sleep restriction

TFA: time-frequency analysis

TSD: total sleep deprivation

TSR: total sleep restriction

VAS: visual analogue scale

Introduction

Nociception, pain and pain perception: A brief overview

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential damage, or described in terms of such damage" (Merskey and Bogduk, 1994). Briefly, pain may be described as "first pain" and "second pain", the former carried to the brain by lightly myelinated A-δ fibres, whereas the latter refers to the slower conduction of pain from unmyelinated C-fibres (Basbaum et al., 2009). Nociception, on the other hand, refers to the process of encoding noxious stimuli (ibid). Thus, it is argued that although nociception and pain are two highly interlinked phenomena, pain and nociception remain two separate entities. Stated differently, a human being may suffer pain in the absence of nociceptive activity, and likewise, nociceptive activity may not necessarily lead to the perception of pain (Melzack and Katz, 2013). As such, pain may be viewed as one of the fundamental human senses with specific, behavioural and motivational incentives and must be investigated accordingly (Craig, 2003).

The introduction to this thesis will first outline three major theories regarding pain and its complexity, then describe the mechanisms underlying expectations of positive and negative meaning and briefly outline the major components of sleep physiology. Finally, I will summarise and review the literature investigating the effects of shift work on pain.

Pain models

There are several pain models attempting to encompass the multifactorial aspects of pain. Moayedi and Davis (2013) present a historical overview of the major influential theories of pain which ultimately culminated in the development of the "Gate Control Theory of Pain". Briefly, Melzack and Wall (1965) proposed that the substantia gelatinosa in the dorsal horn of the spinal cord functions as a "gate keeper", effectively controlling the transmission of nociceptive and other sensory stimuli to higher cortical processing areas [for a complete review, see Mendell (2014)]. Melzack in collaboration with Casey (Melzack and Casey, 1968) then proposed the neuromatrix model of pain, which endeavours to encompass the multidimensional aspects of pain. In this model, the authors refer to a genetically in-build neuromatrix which may be subdivided into sensory-discriminative, affective-motivational and evaluative-cognitive components. Following a higher cortical cyclical process, the

output, referred to as the neurosignature, leads to an individually produced perception of pain (Melzack and Casey, 1968, Melzack, 1999, Melzack and Katz, 2013).

The highly influential work of Melzack, Wall and Casey, triggered research into pain which did not exclusively focus on the nociceptive, peripheral component, but rather focused on the cortical activation patterns involved in the perception of pain. Accordingly, the "pain matrix" was introduced, which constitutes a set of cortical areas involved in processing nociceptive stimuli and pain perception (Ingvar, 1999). The canonical areas are the primary and secondary somatosensory cortices (SI and SII, respectively,) the cingulate cortices and the insular cortices (Ingvar, 1999, Borsook et al., 2010). However, in a seminal review by Legrain et al. (2011), the authors propose that the "pain matrix" is not exclusively responsive to nociceptive stimuli. Rather, they argue that these cortical regions respond to a multitude of stimuli, of which nociceptive stimuli are of great importance due to the ability to stand out from other sensory stimuli. Moreover, they propose that the pain matrix network functions as a salience detection system for the body, allowing potentially dangerous or threatening sensory stimuli to be fast-tracked into behaviourally important responses (Legrain et al., 2011).

Finally, a third model is worth mentioning. Moseley and Vlaeyen (2015) proposed "the imprecision hypothesis of chronic pain" in which the authors consider chronic pain in light of associative learning processes. Drawing on knowledge from the field of associative learning and cognitive neuroscience, the authors make a strong argument for pain as product of cortical activity and not merely nociceptive stimuli. And, importantly, the precision with which nociceptive stimuli are encoded alongside other sensory stimuli, predicts the degree of subsequent pain activity. The more precisely a nociceptive stimulus is encoded, the more "correctly" its associative learning is encoded. Likewise, an imprecise encoding of nociceptive stimuli, may potentially lead to an increase in associative learning taking place amongst the other sensory stimuli present at the time of injury, with the potential for generating widespread "non-specific" pain as a consequence (Moseley and Vlaeyen, 2015). Thus, in light of the increasing understanding of pain perception, it is clear that it is insufficient to view pain as a linear consequence of nociceptive input, but rather as the sum of highly elaborate cortical processes (Melzack and Katz, 2013, Moseley and Vlaeyen, 2015).

Key terminology: Hyperalgesia and allodynia

Two common presentations of pain may serve to illustrate the complexity of pain perception: *Hyperalgesia* refers to an increased sensitivity to painful stimuli and *allodynia* refers to the process of otherwise non-painful stimuli being perceived as painful (Merskey and Bogduk, 1994). Given that pain perception reflects the summed activity of nociceptive input and cortical processing of said input, researchers discriminate between the nociceptive components ("Bottom-Up") vs the cortical modulatory components ("Top-Down") (Gilbert and Sigman, 2007, Legrain et al., 2012). Consequently, hyperalgesia and allodynia explained from a "Bottom-Up" perspective occur as a consequence of increased or magnified nociceptive firing, which may be a result of peripheral sensitisation (Fabrizi et al., 2013) or continuous afferent nociceptive input (Vaso et al., 2014). From a "Top-Down" processing perspective, hyperalgesia and allodynia may occur as a consequence of increased central sensitisation (Latremoliere and Woolf, 2009), altered descending modulation (Lau and Vaughan, 2014) or altered expectation (Hauck et al., 2007b). As a great deal of research has focused on positive expectations (placebo), I will next review the field of pain processing in relation to both nocebo and placebo.

Expectations: Placebo and nocebo

Any medical or research procedures applied to humans do not act solely in isolation, but rather interact with the receiver in complex ways (Benedetti and Amanzio, 2011). Placebo and nocebo are the latin words for "I shall please" and "I shall harm" respectively, and refer to the complex psychosocial context surrounding the patient and the power the brain has to affect bodily sensations and functions (Tavel, 2014). In general, placebo refers to the functional improvement observed in response to an intervention and may be divided into placebo effects and placebo responses (Benedetti et al., 2011). Specifically, the placebo effect relates to any improvement observed in clinical trials that is not related to the drug itself, whereas the placebo response refers to the neurobiological, cognitive processes that shape these improvements (Benedetti et al., 2011). The two terms are used interchangeably in the literature (Benedetti, 2013) and consequently, they will be used as synonyms throughout this thesis.

The nocebo effect is considered opposite to the placebo effect (Jakovljevic, 2014). It too may be divided into a nocebo effect and a nocebo response. The former refers to the

negative psychosocial context surrounding the patient and treatment not related to the drug itself, whereas the latter refers to the neurobiological, cognitive processes involved in shaping these responses (Benedetti et al., 2007). Finally, Moerman (2011) makes a strong argument for substituting the terminology *placebo response* with *meaning response*. Moerman (2011) argues that a placebo drug indeed does nothing, however, meaningful words and meaningful utterances presented alongside the placebo drug lead to powerful responses. Consequently, the focus should be on the contextual settings, or meaning, framing the delivery of the placebo.

Placebo-nocebo: Neuropsychology and neurobiology

Placebo-nocebo responses involve many regions and components of the nervous system, such as the endocrine system (Price et al., 2008a), pain modulatory system (Benedetti and Amanzio, 2011) and learning and memory (Benedetti et al., 2011). It is however, best studied in subjective phenomena such as pain perception (Tavel, 2014). Understanding how placebo-nocebo mechanisms may be mediated in pain perception is of great interest and several theories have been proposed. The influence of expectation is investigated by Colloca et al. (2008) and Koyama et al. (2005).

In a nocebo procedure involving verbal instruction preceding either tactile non-painful stimuli or low intensity painful electrical stimuli (Colloca et al., 2008), healthy volunteers exhibited allodynic responses to the tactile stimulation, and hyperalgesic responses to a low intensity painful stimuli, subsequent to nocebo suggestions of a negative outcome. Thus, the authors argue that expectations of a negative outcome adversely modulate the perception of the tactile and painful stimuli.

Likewise, placebo analgesia is seen to occur when pain reduction is expected (Koyama et al., 2005). In an experiment using thermal noxious stimuli, Koyama et al. (2005) investigated how expectations of forthcoming painful stimuli modulated the subsequent subjective pain perception and cortical activation using functional magnetic resonance imaging (fMRI). Interestingly, expectations of increased pain did not significantly alter the subjective experience of the painful stimuli, however, expectations of decreased pain profoundly affected the subjective rating of pain and the activity of typical pain related cortical areas. Most notably, the SI, SII, anterior cingulate cortex (ACC), pre-frontal cortex (PFC) and the cerebellum showed consistent activity and the positive expectation of reduced pain

produced a reduction in pain perception rivalling that obtained from a standard dose of morphine (Koyama et al., 2005). Recently, a study by Zeidan et al. (2015) using large discrepancy between expected and actual experimental pain confirmed activity in the same cortical areas reported by Koyama et al. (2005), but also added that the posterior parietal cortex (PCC) is involved.

Placebo-nocebo: Neurophysiology

Two possible avenues for placebo-nocebo mediated hyperalgesia and hyperanalgesia are particularly worth exploring. According to a recent review by Colloca and Grillon (2014), placebo-nocebo act upon the endogenous release of opioids and cholcystokinin (CCK), thereby facilitating placebo analgesia and nocebo hyperalgesia, respectively. In a pioneering study by Levine et al. (1978), the effect of naloxone, a known opiate receptor blocker was tested on post-operative dental pain. The patients were randomly assigned to morphine, placebo or naloxone. Pain intensity was measured using a visual analogue scale (VAS). The patients were further subdivided into placebo responders and non-responders. The results indicated that naloxone clearly reduced the placebo effect as indicated by higher experienced pain levels in the placebo responders compared to the non-responders. Additionally, when naloxone was administered prior to morphine as opposed to after morphine, the probability of obtaining a placebo response was reduced (Levine et al., 1978). The authors conclude that endorphins (endogenous opioids) activity account for the observed placebo analgesia, which has been supported by subsequent findings (Benedetti et al., 2007).

The effects of CCK seem to oppose that of opioids, with results indicating a nocebo-induced hyperalgesic effect. In a clinical study by Benedetti et al. (1997), post-operative patients were treated with proglumide, a CCK non-specific antagonist. Proglumide was found to prevent nocebo hyperalgesia in a dose-dependent manner, indicating that nocebo-induced hyperalgesia is mediated, at least partly, by CCK. To add further support for the role of CCK in nocebo hyperalgesia, another study by Benedetti et al. (2006) investigated the effect of verbal suggestions of hyperalgesia in ischaemic arm pain. Measurements of adrenocorticotropic hormone (ACTH) and cortisol plasma levels concentrations were made to assess the involvement of stress and anxiety, by way of the hypothalamic-pituitary axis (HPA). Interestingly, both nocebo-induced hyperalgesia (NIH) and HPA hyperactivity were

blocked by diazepam, (anti-anxiety drug), however, proglumide had a dinstinct effect on nocebo-induced hyperalgesia yet no effect on HPA activity. The authors concluded that CCKs have a specific role in mediating NIH (Benedetti et al., 2006). Thus, it has been argued that the opioidergic and the CCKergic systems have opposing roles, the former being activated by positive suggestions leading to placebo-induced analgesia, whereas the latter is seen to be activated by negative suggestions, leading to NIH (Benedetti et al., 2007).

Recent studies point to additional mechanisms involved in placebo-nocebo effects. Geuter & Buchel (2013) investigated the effects of nocebo on cervical spinal cord activity in response to heat pain. Using fMRI, they found that healthy volunteers exposed to a nocebo cream believed to contain capsaicin, exhibited increased activity in the ipsilateral dorsal horn of the spinal cord, corresponding to the C5/C6 dermatome. Moreover, they compared the activity to VAS scores and pain threshold and argued that 'top down' processing occurred at spinal level. However, the degree to which supraspinal versus spinal processes are involved remains unclear (Geuter and Buchel, 2013).

Electroencephalogram (EEG)

Traditionally, measures of brain activity with electroencephalography (EEG) are used to study changes in brain activities that are time and phase-locked to sensory, motor or cognitive events (Kalcher and Pfurtscheller, 1995). These changes in brain activity are referred to as event related potentials (ERPs) and are thought to represent a summation of time-locked dipoles generated by post synaptic potentials (Sur and Sinha, 2009). By time-averaging over repeated trials, it is argued that this improves the signal-to-noise ratio (Luck, 2014). However, in doing so, there is a risk of missing a considerable amount of data, as ERPs that are not perfectly time and phase locked to the stimulus may go undetected due to jitter (Pfurtscheller and Lopes da Silva, 1999). Consequently, it is argued that other means of investigating the information flow is more appropriate when dealing with subjective phenomena such as pain perception (Mouraux and Iannetti, 2008, Schulz et al., 2011).

One such means is time-frequency analysis (TFA), in which event-related phenomena are due to frequency specific changes of the ongoing EEG activity (Pfurtscheller and Lopes da Silva, 1999). Generally, this may represent an increase of power in a given frequency band (synchronisation) or a decrease in power in a given frequency band (desynchronisation), and is referred to as "event-related synchronisation" (ERS) and "event-related

desynchronisation" (ERD), respectively (Pfurtscheller and Lopes da Silva, 1999, Mouraux and Iannetti, 2008). ERS-ERDs may thus be viewed as alterations in the parameters that control oscillations in neuronal networks (Pfurtscheller and Lopes da Silva, 1999), and may shed light on the complexity underlying subjective phenomena, such as pain perception.

It is worth pointing out however, that the increase or decrease in EEG oscillation power represents the activity of a population of neurons within a given frequency band, and not an overall increase or decrease of single-neuron activity (Mouraux and Iannetti, 2008). Neural oscillations are characterised by their frequency, amplitude and phase and are commonly divided into alpha (8-13 Hz), beta (13-30 Hz), gamma (> 30 Hz), delta (< 4 Hz) and theta (4-8 Hz) in humans (Luck, 2014).

EEG and expectations

Regarding the effects of expectations on EEG activity, very little is known. Lorenz et al. (2005) investigated the effects of positive and negative expectations on pain intensity using a combined magnetoencephalogram (MEG) and EEG procedure. They found a strong association between the signalled intensity and the perceived intensity, that is, the placebo procedure yielded less pain from a high intensity stimulus. Likewise, the nocebo procedure led to more pain from a low intensity stimulus. Using source-localisation, they identified the peak amplitude of the MEG signal to occur in the SII and the peak amplitude of the EEG signal in the ACC (Lorenz et al., 2005). However, they did not investigate the time-frequency components and the event-related peaks may likely reflect the detection of a salient stimulus as opposed to actual coding of the painful stimuli (Mouraux and Iannetti, 2009).

Using TFA, Huneke et al. (2013) investigated the effect of a placebo procedure upon resting state alpha oscillations and found that subsequent to the placebo, the alpha activity increased significantly compared to the control group. Using LORETA as a means for source localisation, the authors argue that the observed increase in alpha activity may be generated in the dorsal ACC, medial pre-frontal cortex (mPFC) and the insula.

Recently, Tiemann et al. (2015) investigated painful thermal stimuli and the effect of expectations, by way of a placebo procedure and EEG activity. They reported a significant effect of stimulus intensity and placebo on event related potentials (ERPs) and in the theta frequency band representing pain-induced responses. However, there were no findings

regarding the placebo effect in the remaining frequency bands, specifically, the alpha and gamma band. The gamma band activity is of particular interest, as some authorities claim that the extent of gamma band oscillations (GBOs) may be highly reflective of the actual cortical network involved in the multidimensional encoding of pain (Gross et al., 2007, Schulz et al., 2011). Additionally, Zhang et al. (2012) argue that GBOs are highly indicative of the subjective pain intensity and lie at the interface between stimulus-driven and cortical modulatory determinants of pain perception. However, the extent to which GBOs or other specific frequency bands are involved in sleep induced hyperalgesia (SIH) remain largely unknown, and thus, serves as an indication for the present study.

The effects of expectations and how they may interfere with pain perception through sleep restrictions, is even less clear. Laverdure-Dupont et al. (2009) propose an interesting model in which the amount of REM sleep affects subsequent expectancy-mediated processes. Specifically, the authors propose that reduced REM sleep is associated with a facilitation of expectancy-mediated responses, arguing that the mechanisms may be related to sleep-induced learning processes. However, the literature regarding how sleep restrictions may potentially interact with nocebo and subsequent pain perception, is to my knowledge absent, and serves as a major focus of this study.

Sleep physiology: A brief overview

Humans spend approximately one-third of our lives sleeping, which has been described as a state of immobility with greatly reduced responsiveness, yet readily reversible (Siegel, 2005). Specifically, sleep may be divided into two main phases: rapid eye movement sleep (REM) and non-REM (NREM) sleep (Porkka-Heiskanen, 2013). REM sleep is characterised by its almost complete lack of muscle tone due to inhibition of the spinal motor neurons by descending pathways (Kandel et al., 2013). Non-REM sleep may be subdivided into four additional stages, with stage 1 representing light sleep, stage 2 and 3 characterised by sleep spindles and stage 4 representing deep sleep. Stage 4 sleep is characterised by high-voltage, slow wave (0.5-4 Hz) activity (Saper et al., 2010). A sleeping person normally displays several cyclical transitions between light and deep sleep and subsequent REM sleep, in which the REM phase becomes progressively longer during the night (Saper et al., 2010, Kandel et al., 2013). Furthermore, Saper and colleagues have proposed a “flip-flop” switch system, in

which mutually inhibiting cortical circuits allow for swift transitions between awake and sleep, and transitions between NREM and REM sleep (Saper et al., 2001, Saper et al., 2005).

The purpose of sleep is intimately linked to sleep homeostasis (Porkka-Heiskanen, 2013). Briefly, a period of wakefulness is followed by a period of sleep, and ultimately the sleep propensity, or urge to sleep arises from the length of waking. According to Porkka-Heiskanen (2013), three main theories of sleep function dominate: Metabolic, synaptic and immunological models, which will be briefly outlined below:

The *energy metabolism theory* basically proposes that prolonged periods of wakefulness lead to energy depletion, and, importantly, sleep allows for restoration of used metabolites (Benington and Heller, 1995). The *synaptic homeostasis theory* argues that synaptic strengthening and neural plasticity take place during waking, and are subsequently maintained or regulated during various sleep stages (Tononi and Cirelli, 2006). Lastly, prolonged wakefulness may potentially activate certain components of the immune system and sleep may thus serve an important immunological purpose (Krueger et al., 2011).

Sleep related problems

The purposes of sleep may be further studied by examining the detrimental effects of various sleep disorders. An increasing body of knowledge indicates that sleep disorders are associated with a variety of conditions, such as coronary artery disease (Mallon et al., 2002), hypertension (Suka et al., 2003) and chronic pain (Kundermann et al., 2004). According to Mahowald and Schenck (2005), most sleep complaints fall into four categories: Hypersomnia, insomnia, circadian rhythm disorders (CRD) and parasomnias. *Parasomnias* refer to undesirable behavioural phenomena that occur during sleep, such as sleepwalking or sleep terrors, whereas *CSD* refer to problems with sleeping in accordance with the desired light-dark cycle (Mahowald and Schenck, 2005). *Hypersomnia* refers to excessive daytime sleepiness without obvious explanation, and is intimately linked to insomnia (Kandel et al., 2013). Whereas hypersomnia often stems from volitional sleep deprivation, *insomnia* is the most prevalent sleep complaint in the general population, and refers to the trouble of falling or staying asleep (Mahowald and Schenck, 2005). It has been estimated by Morin et al. (2009) that as many as 30% of the adult population report symptoms of insomnia and between 6-10% meet diagnostic criteria for an insomnia disorder. Likewise, it is estimated

that between 10-20% of the adult population suffer from some form of chronic pain of moderate intensity (Breivik et al., 2006, Mundal et al., 2014).

The incidence of fibromyalgia, a condition characterised by both poor sleep patterns and chronic pain (Bigatti et al., 2008), has been estimated to reside between 3-5% of the population (Gran, 2003). Longitudinal studies have described a strong dose-dependent association between sleep problems and risk of fibromyalgia (Mork and Nilsen, 2012) and sleep problems and risk of chronic pain (Sivertsen et al., 2015). Additionally, Lallukka et al. (2014) reported a synergistic interaction effect of insomnia and pain and subsequent disability retirement. It is however, unclear whether sleep restriction leads to increased prevalence of pain, or chronic pain leads to altered sleep pattern. In a recent review, McBeth et al. (2015) argue that the relationship is indeed bi-directional, whereas Finan et al. (2013) on the other hand, make a strong argument for insomnia as a major factor in the development of pain, but do not seem to find the same support for pain leading to insomnia.

Sleep and shift work

It is not surprising then, that shift workers and particularly those working night shifts are prone to a variety of health issues, including chronic pain. Zhao et al. (2012) studied the effects of shift work on nurses and found that shift work increased the risk of developing low back pain (LBP) by as much as 40%. This has been supported by Buja et al. (2013) who found higher levels of self-reported gastrointestinal and stress related symptoms, particularly LBP, in nurses working nightshifts. Additionally, Barro et al. (2015) reported a high prevalence of musculoskeletal pain in shift workers at a poultry factory and that the prevalence increased in night shift workers and length of night shift employment. Recently, Takahashi et al. (2015) investigated the effects of night shifts longer than 16 hours and the relationship with low back pain and perceived sleep problems in factory workers. Their findings indicate that extended night shifts are associated with disabling LBP, moreover, if the participants identified additional sleep related problems, the association between nightshift and disabling LBP increased. Contrary to these findings, Mehrdad et al. (2012) studied Iranian physicians and found that their prevalence of musculoskeletal complaints was less than that of comparable health workers and levelled that of the general population. As the vast majority of research in the field of shift work relies on qualitative measures (self-reported

outcome measurements), there is a need to research this experimentally, using quantitative measures such as EEG.

Experimental findings seem to support the notion that sleep restrictions lead to hyperalgesia. In an experimental study, Schuh-Hofer et al. (2013) found that following one night of total sleep restriction (TSR), the participants showed hyperalgesic responses to several stimuli such as heat, mechanical pain and pinprick. Similar experimental findings have been reported by Ødegard et al. (2015) who reported sleep induced hyperalgesia (SIH) accompanied by a reduction in laser evoked potentials (LEPs). The authors propose that SIH may be caused by perceptual changes rather than sensory amplifications.

In another experimental study using painful electrical stimuli in healthy volunteers exposed to two nights of 50% sleep reduction, Matre et al. (2015) also reported SIH and specific changes in EEG activity. Notably, ERPs were not altered due to sleep restrictions. However, the authors report specific changes in the TFA, including sleep-induced ERS observed at Cc electrode and sleep-induced ERD in the alpha bandwidth. The authors propose that the observed cortical changes following sleep restrictions may potentially reflect reduced cortical processing in the somatosensory cortex (Matre et al., 2015). Lastly, a recent meta-analysis by Schrimpf et al. (2015) maintains that experimental sleep restrictions lead to hyperalgesia and argues that there is a need to extend these findings into clinically relevant studies, which serves as an indicator for the present study.

Pain, nocebo, sleep, EEG and shift work: In summary

Pain perception is multifactorial and must be investigated accordingly. Experimentally induced sleep restrictions lead to hyperalgesia. Positive and negative expectations influence pain perception, however, nocebo and the potential role in sleep induced hyperalgesia remains elusive. Long term sleep related problems are associated with increased risk of pain and there are indications that shift work is associated with increased prevalence of pain conditions. The evidence from self-reported studies is however, not conclusive, highlighting a need to investigate shift workers and pain perception using quantitative methods. Recent findings indicate that more sophisticated EEG measurements, such as time-frequency analysis may shed light on the cortical mechanisms underlying sleep induced hyperalgesia, nocebo and pain perception.

Aims

- To investigate if night shift work (NSW) leads to altered pain perception in a cohort of nurses exposed to experimentally-induced electrical pain stimuli.
- To investigate if NSW leads to altered pain-elicited cortical responses in the time-frequency domain, in a cohort of nurses.

Hypotheses

- Following NSW, the participants will exhibit an increase in pain scores, as measured by a VAS.
- Following nocebo, the participants will exhibit an increase in pain scores, as measured by a VAS
- Sleep-induced hyperalgesia is facilitated by negative expectations (nocebo).
- SIH will be accompanied by altered magnitude in specific time-frequency responses in the delta, theta, alpha and gamma bandwidth.
- Nocebo will be accompanied by altered magnitude in specific time-frequency responses in the delta, theta, alpha and gamma bandwidth.

Methods of Investigation

Subjects

57 nurses were recruited through poster advertising and flyers distributed at hospitals and certified health clinics. Following an initial assessment, 53 nurses, 41 females and 12 men, (mean age 31.6 ± 9.06, range 24-57) were included and completed the study. The nurses were included in the study if they worked in a rotating shift schedule in a minimum 50% position, including night shifts. Four participants opted to withdraw from the study following the initial consultation. A further 11 participants withdrew from the study between Day 1 and Day 2 and additionally two participants were excluded due to pregnancy. Consequently, the dataset is slightly unbalanced, with a larger proportion of females than men and a larger cohort from Day 1 compared to Day 2. However, the dataset contains 44 recordings following HS and 47 recordings following NSW, providing a balanced dataset regarding the sleep condition. Further, the participants were instructed to refrain from alcohol and over-the-counter analgesics 24h before the experiments. The participants were informed of the primary purpose of the study group, which was to investigate the potentially negative health effects of shift work. They were however, blinded to the specific hypotheses concerning this particular study. The participants received a small financial imbursement (NOK 1000) for participating in the study. *Figure 1* provides an overview of the experimental process, from recruitment through to the statistical analysis:

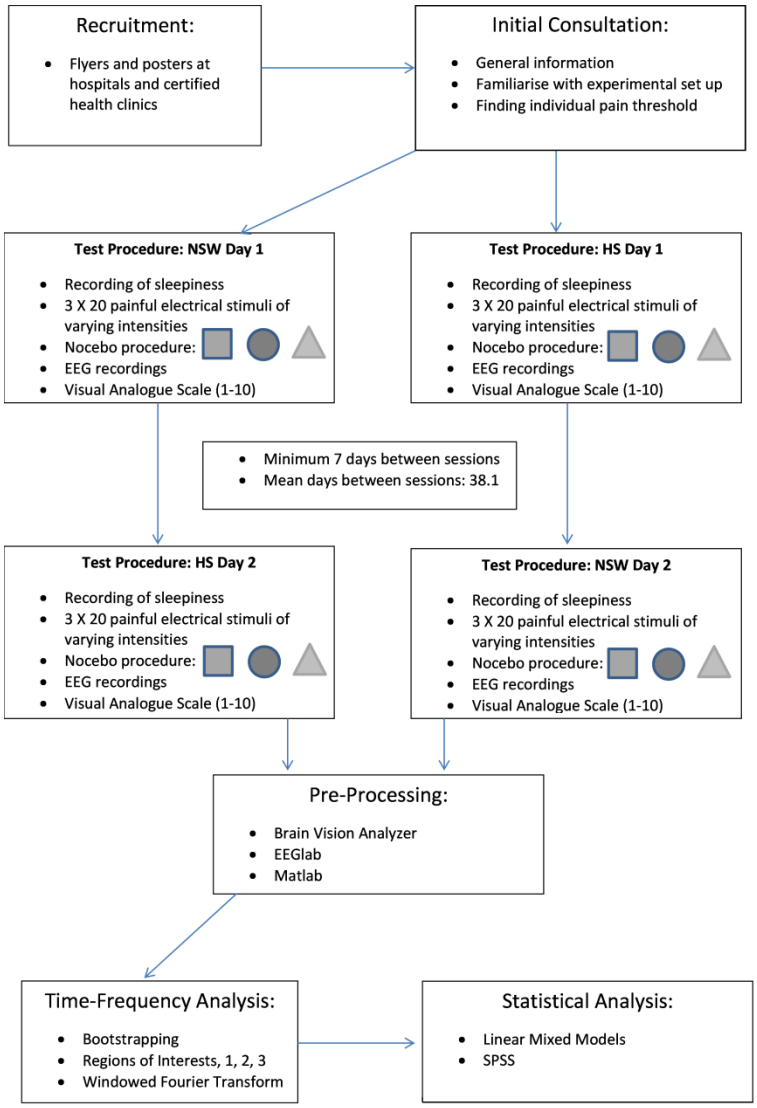

Figure 1: A schematic overview of the experimental process. The participants were recruited primarily from hospitals. At the initial consultation, the participants were introduced to the experimental setting and individual pain thresholds were set. The participants were then included in a paired cross-over study in which they received the same experimental procedure twice, after two consecutive nights of HS and following two consecutive nights of NSW. The EEG data were then subsequently pre-processed in Analyzer, EEGlab and Matlab. The time-frequency analysis was performed in Matlab and the statistical analysis was performed in SPSS using Linear Mixed Models.

Design

The study design was a paired cross-over in which the participants received the same protocol under two different conditions. The study was approved by the Norwegian Regional Committee for Medical Research Ethics (Approval number: 2012/199)

Procedure

Subjective sleepiness was measured using Karolinska sleepiness scale (KSS) at the start of each experiment (Akerstedt and Gillberg, 1990). Additionally, behavioural alertness was measured using a computerised version of the psychomotor vigilance test (PVT) (Basner and Dinges, 2011).

Electrical pain stimulation:

The participants received painful electrical stimuli delivered to the anterior aspect of the forearm, through a platinum electrode (diameter 0.2 mm) placed approximately 10 mm medially to half the distance between the insertion of the biceps brachii tendon and the distal end of the ulna. The pin electrode served as the cathode and the anode was a conductive Velcro-Strap (Alpine Biomed ApS, Skovlunde, Denmark) which had been soaked in an isotonic NaCl solution and placed on the ipsilateral belly of the biceps brachii muscle, 5 cm proximal to the cubital fossa. A constant current stimulator (DS7A and DG2A, Digitimer, Hertfordshire, U.K) delivered each electrical stimulus as a double-pulse, in which each pulse lasts 0.5 ms and is separated by 10 ms, ensuring that the two pulses are perceived as one single pulse. The conduction velocity is compatible with the activation of Aδ-fibres (Tran et al., 2008).

The pain threshold (PT) was set individually by using a ladder sequence of three ascending series of stimuli. Each series started at 0 mA and progressively increased by 0.1 mA until the lowest mA value perceived as painful by the participant. The PT was then calculated as the mean of the last two mA values. The painful electrical stimuli were then randomly delivered at three different intensities. S*timulus Intensity A* equaled two times PT, S*timulus Intensity B* equaled three times PT, and finally, S*timulus Intensity C* equaled four times PT. In order to investigate the effect of expectations on pain perception, each stimulus was preceded by a warning signal, indicating the intensity level of the impending stimulus. Thus, Stimulus Intensity A was indicated by a square, Stimulus Intensity B by a circle and Stimulus Intensity C by a triangle. In order to introduce negative expectations (nocebo), the stimulus was signalled as higher than the actual intensity delivered. Consequently, in the nocebo procedure, Stimulus Intensity A was preceded by a circle (indicating Stimulus Intensity B) and Stimulus Intensity B by a triangle (indicating Stimulus Intensity C). There was no nocebo condition for Stimulus Intensity C. Thus, the participants received a total of 60 electrical stimuli; 20 correctly signalled stimuli A and B, 20 correctly signalled stimuli C and 20 stimuli that were signalled as intensity B and C, but were actually delivered as intensity A and B, respectively.

The participants were asked to rate the pain intensity following each electrical stimulus. An electronic version of a VAS was used (0-10 cm), ranging from “0” (no pain) through “10”

(most intense pain imaginable). The participants were instructed to rate the pain intensity 3-4 seconds after each stimulus. The pain scores were then averaged across trials and abbreviated as "VAS_mean" in the subsequent analysis. The electronic VAS has been found to be a reliable and valid tool for measuring pain intensity in experimental settings (Price et al., 2008b).

EEG- recordings

EEG measurements were recorded from 32 electrodes placed according to the international 10-20 system (actiCAP, Brain Products GmbH, Gilching, Germany). The continuous EEG data were pre-processed in Brain Vision Analyzer and EEGlab , which included downsampling to 512 Hz, re-referencing to linked mastoid (electrodes TP9 and TP10), eye blinks and ocular movements correction by Independent component analysis based on the upper left (VEOG) and lower right (HEOG) side of the eye and filtering (0.53-100 Hz). The data were sampled at 2 kHz and impedance was kept below 20 kΩ. The trials were then split into epochs of 2500 ms and exported to Matlab. Lastly, the data were manually inspected and segments with artefacts were removed (cut-off 100 mV). EEG data were analysed from 10 electrodes (see *Figure 2*). Responses from contralateral responses were evaluated, in line with previous findings regarding gamma and alpha activity (Gross et al., 2007, Hauck et al., 2007a, Zhang et al., 2012, Matre et al., 2015). Thus, as an example, F3/4c constitutes the cortical activity of right arm stimulation measured at F3 and left arm stimulation measured at F4 electrode.

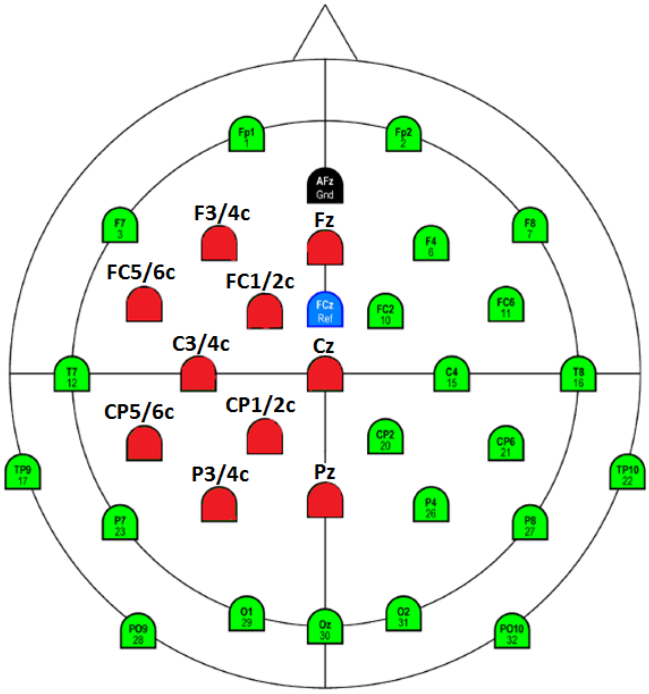

Figure 2: Overview of the electrodes that were investigated (marked in red). Responses from paired electrodes (F3/4c, FC1/2c, FC5/6c, C3/4c, CP1/2c, CP5/6c and P3/4c) were arranged so that contralateral responses were evaluated. Consequently, F3/4c constitutes the activity of responses at F3 electrode from stimulation of the right forearm, and responses at F4 electrode following stimulation of the left forearm. Black and blue electrodes represent ground electrode and reference electrode, respectively. Note: Oz and O1/2c electrodes were included in the ROI 3 analysis.

Time Frequency Analysis:

The TFA was performed in Matlab using custom written Matlab scripts (Matre et al., 2015), however, the TFA procedure is based on the parameters outlined by Zhang et al. (2012). A Windowed Fourier Transformation (200 ms Hanning window) was applied at each epoch and averaged across trials. This allows for capturing activity that is phase locked and non-phase locked to the stimulus (Pfurtscheller and Lopes da Silva, 1999). The magnitude of event-related (ER) changes in oscillation amplitude was expressed as a percentage change in power from a pre-stimulus reference interval. The pre-stimulus reference interval was set to -900 ms to -100 ms. The percentage change in oscillation amplitude was expressed as follows:

$$ER\%(t,f) = [P(t,f) - R(f)] / R(f) \times 100$$

$P(t,f) = |F(t,f)|^2$ defines the spectral density at each time-frequency point. R(f) defines the average power spectral density for each subject and condition within the pre-stimulus reference interval, a process that was implemented for each condition (Sleep, Expect and Intens).

Introducing a cognitive task leads to an α-ERD (Lopes da Silva, 2013). To ensure that there was no "floor-effect" (Field, 2009) that could potentially interfere with the subsequent data analysis, it was decided to compare the α-ERD with a secondary reference area, obtained from a time-interval prior to the warning signal. This is referred to as pre-warning, and refers to the time interval (-900 ms - -100 ms) prior to the warning signal. *Figure 3* depicts a schematic overview of the experimental set up:

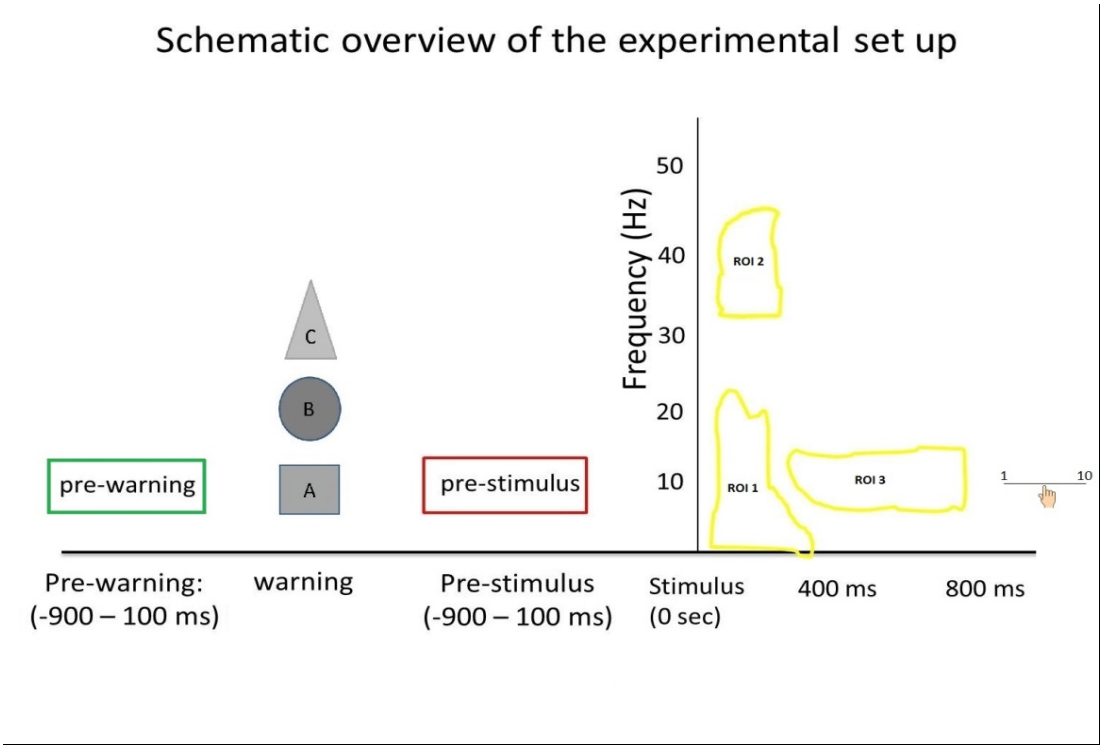

Figure 3: A schematic overview of the experimental set up. Region of interest (ROI) 1, ROI 2 and ROI 3 are encircled in yellow and reflect event-related, frequency-dependant percentage change in oscillation magnitude, relative to a pre-stimulus baseline interval (encircled in red). ROI 3 was further compared to a secondary baseline reference, "pre-warning", encircled by green. The intensity of the impending stimulus was signalled by a square, circle and triangle, indicating stimulus intensity A, B and C, respectively. Pain scores were recorded 3-4 sec after each stimulus on an electronic VAS.

Regions of Interest

Following the TFA, regions of interests (ROIs) were determined using a bootstrapping procedure and paired t-test. Bootstrapping is a statistical technique using random sampling and replacements to infer accuracy of the data (Field, 2009). The TF data were bootstrapped 1000 times before the paired t-test compared the TF-points from the post-stimulus interval (0-800 ms) to the TF-points from the pre -stimulus interval (-900 to -100 ms). The significance level was set to $p < 0.05$. Three ROIs were identified and included in the statistical analysis.

ROI 1: ERS in the 1-400 ms/1-25 Hz post stimulus interval, with a maximum power at approximately 200-400 ms post stimulus.

ROI 2: GBOs in the gamma frequency range (approx. 35-85Hz) with a maximum power at approximately 100-200 ms post stimulus.

ROI 3: ERD in the alpha bandwidth (8-12 Hz) with a maximum power at approximately 400-500 ms post stimulus. *Figure 4* depicts an actual time-frequency recording with ROI 1, ROI 2 and ROI 3.

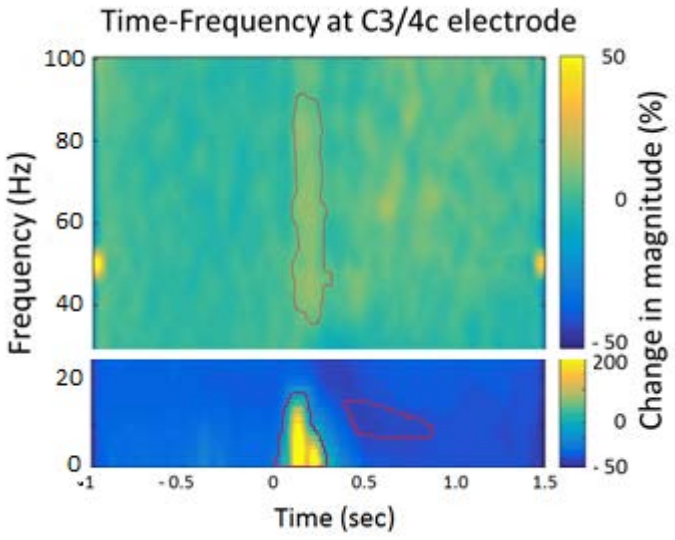

Figure 4: Overview of an actual time-frequency recording at C3/4c electrode. The plot is split in two, for descriptive purposes. The numbers on the left y-axis indicate the frequency band (Hz). The numbers on the right of the figure indicate percentage change in oscillation power compared to a pre-stimulus reference area. The x-axis represents time, in which 0 represents the time of the delivery of the painful electrical stimulus. Note: The ROIs are encircled for illustrative purposes. Slight differences in ROIs were observed between various electrodes.

Statistical Analysis

The data were exported to SPSS for statistical analysis (IBM SPSS version 21, Chicago, Illinois, USA). To ensure that the researcher remained blinded to the sleep conditions, the data files were recoded by numbers. Thus, each trial consisted of 7 conditions: Three conditions representing *Stimulus Intensity A, B and C (Abbreviated as "Intens"* A, B, C). Two conditions representing *Expectations,* (Abbreviated as "Expect", A = Correct Signalling, B = Nocebo) and finally two conditions representing *Sleep Condition* (Abbreviated "Sleep", HS = Habitual Sleep and NSW = Night Shift Work).

Electrophysiological and psychophysical measurements were analysed using linear mixed models (LMMs), maximum likelihood (ML) and restricted estimation maximum likelihood (REML) criteria. In a comparison between LMMs and traditional repeated measures of

ANOVA in EEG research, it was argued that LMMs hold several advantages (Vossen et al., 2011). Among a few, the most important ones are the ability to include single trial data and include individual differences in the within-subject variances (Vossen et al., 2011).

The data were checked for outliers in which responses that were higher or lower than 3 x standard deviations were filtered from the data pool. Residuals were plotted as histograms and visually inspected for normality during the statistical analysis. In the present study, dependent variables were subjective pain scores (VAS_Mean), pre-stimulus α-level and electrophysiological data from the electrodes which were analysed in the time-frequency domain (ERS, GBOs, α-ERD). The pre-stimulus α-level is known to fluctuate according to attention and subsequently affect neural responses (Ploner et al., 2006), therefore LMM was performed for pre-stimulus α-level first and included Sleep, Expect and Intens as fixed factors. Subsequently, LMM was performed for the mean pain score and each electrode and included the same fixed factors (sleep, expect and intens). In order to find the optimal model with ML, random "INTERCEPT" was included in the model if it improved the Bayesian Information Criteria (BIC). Likewise, the interaction between "sleep" and "expect" conditions (Sleep x Expect) and "sleep" and "intens" conditions (Sleep x Intens) was included if it improved the model. REML was added prior to the final statistical analysis. In order to control for multiple testing, false-discovery-rate correction (FDR) was performed as described by Benjamini and Hochberg (1995). The level of significance was set at $p = 0.1$.

For the analyses of main effects of sleep and expect conditions, it was decided to remove Stimulus Intensity C data from the analyses, as Stimulus Intensity C did not contain a nocebo procedure. For the ROI 2 analyses all intensity levels were included, as one of the main outcome measures relates to GBOs and the interaction between GBOs and stimulus intensity level. Additionally, after performing a paired samples t-test comparing stimulus intensity C with a random selection of 10 stimulus intensity C, it was decided to keep 20 stimuli in the stimulus intensity C condition, as there were no differences observed in the mean activity level at Cz electrode ($p = 0.43$). The effect of gender was not included in the statistical model due to uneven contribution of women and men (41 vs 12, respectively).

Results:

Psyhophysical measurements

Sleepiness:

The participants were significantly more sleepy following NSW compared to HS (NSW = 6.98 ± 1.1 vs HS = 4.23 ± 1.7, p = 0.001) and the PVT response speed was significantly slower following NSW (NSW 2.44 ± 0.6 vs HS = 2.60 ± 0.4, p = 0.025).

Pain scores:

The results are presented as estimated marginal means ± standard error (EMM ± std err). Following NSW, the subjects rated the electrical stimuli as significantly more painful (F (1, 36) = 9.86, p = 0.003) than following HS (2.9 ± 0.2 vs 2.4 ± 0.2 cm) [Figure 5]. Following nocebo, the subjects rated the electrical stimuli as more painful than following correctly signalled stimuli (nocebo 2.9 ± 0.2 vs correct 2.5 ± 0.2 cm), which was statistically significant (F (1, 86) = 22.04, p = 0.001). The mean pain scores increased in response to increased stimulus intensity (1.7 ± 0.2, 2.6 ± 0.2 and 3.7 ± 0.2) for intensity levels A, B and C, respectively), which was statistically significant (F (1, 163) = 178.25, p = 0.001). There was no interaction between sleep and nocebo condition (F (1, 156) = 0.61, p = 0.438) [Figure 6], and no interaction between sleep and stimulus intensity condition (F (2, 95) = 0.90, p = 0.409). There was a borderline significant effect of age on pain score, indicating an increase in pain scores with increasing age (F (1, 37) =3.86, p = 0.057).

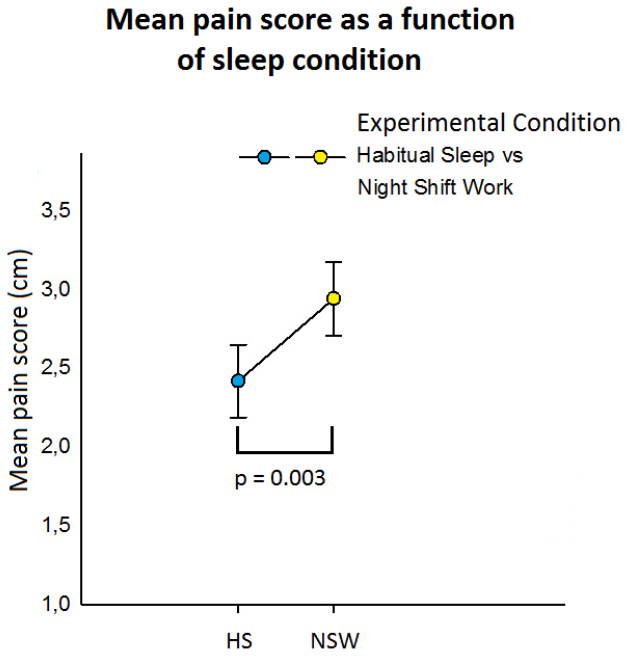

Figure 5: Effect of sleep condition on mean pain score. The y-axis represents mean pain score expressed as estimated marginal means (EMM ± std err). Following NSW, the subjects rated the electrical stimuli as significantly more painful than following HS (p = 0.003).

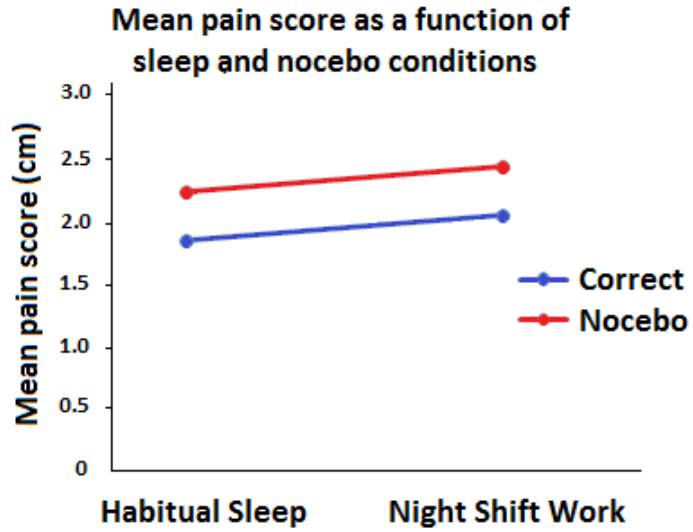

Figure 6: Interaction between sleep and nocebo condition on mean pain score. The y-axis represents mean pain score expressed as estimated marginal means (EMM ± std err). The graph shows that the mean pain score increases for both correct (blue) and nocebo (red) condition in response to NSW, but there is no interaction (p = 0.438).

Electrophysiological measurements

ROI 1: Event-related synchronisation

There was a significant main effect of sleep condition observed at CP1/2c and P3/4c ($p = 0.025$ and $p = 0.007$, respectively), in which the ERS was consistently larger following NSW compared to HS (see Table 1). When correcting for FDR, there was a significant main effect of sleep condition observed at 8 electrodes ($p < 0.05$). *Figure 7* displays a topographic overview of the distribution of the main effect of sleep condition.

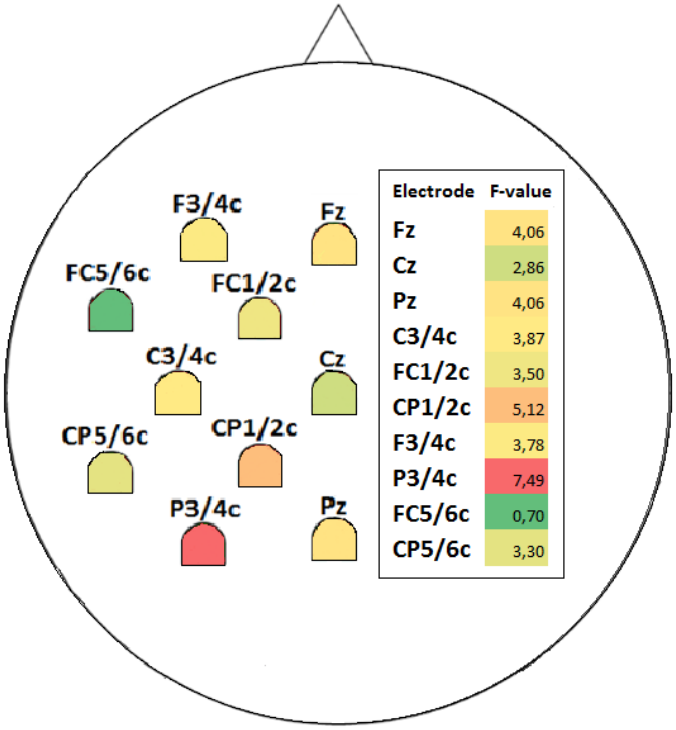

Figure 7: Topographic overview of the level of event-related synchronisation (ERS) observed at various electrodes in response to painful electrical stimuli following NSW. The numbers are expressed in F-values and indicate the statistical effect of NSW on ROI 1 cortical activity. The largest effect is seen at P3/4c electrode and the smallest effect is seen at FC5/6c electrode.

There was no interaction between sleep and expect condition ($p > 0.182$) and no interaction between sleep and intensity condition ($p > 0.147$) observed at any of the electrodes. *Figure 8* presents an overview of the ERS magnitude comparing NSW to HS:

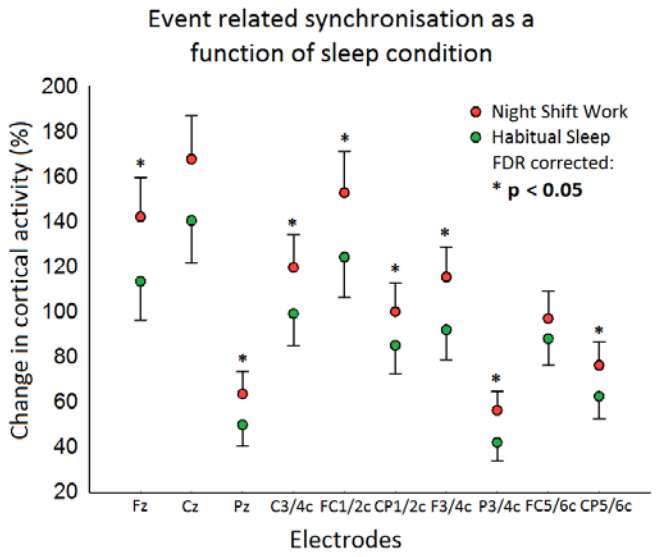

Figure 8: Overview of the level of ERS as a function of sleep condition. The y-axis represents the percentage increase in power relative to baseline in response to painful electrical stimuli (EMM ± std err). The x-axis displays the various electrodes that were investigated. The ERS was consistently larger at all electrodes following NSW (red) compared to HS (green) and was significant at $p < 0.05$ level at 8/10 electrodes following FDR correction (indicated by *).

There was a significant main effect of expect condition observed at Cz, C3/4c and CP1/2c electrodes ($p = 0.010$, 0.022 & 0.005, respectively), in which the ERS was consistently smaller following a nocebo procedure compared to correctly signalled stimuli (see Table 1). Correcting for FDR did not alter the number of significant findings. *Figure 9* presents an overview of the ERS comparing nocebo to correctly signalled stimuli.

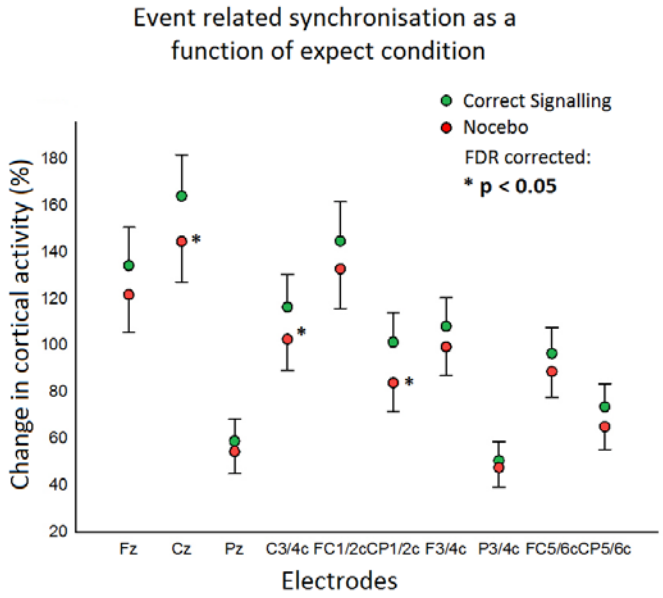

Figure 9: Overview of the level of ERS as a function of expect condition. The y-axis represents the percentage increase in power relative to baseline in response to painful electrical stimuli (EMM ± std err). The x-axis displays the various electrodes that were investigated. The ERS was consistently smaller at all electrodes following nocebo (red) compared to correctly signalled stimuli (green) This was significant at $p < 0.05$ level at Cz, C3/4c and CP1/2c electrodes following FDR correction (indicated by *).

Lastly, there was a significant main effect of stimulus intensity condition observed at C3/4c, F3/4c and FC5/6c electrodes, ($p < 0.05$). However, following FDR correction, no electrodes were significant at $p < 0.05$ level. When assessing the individual electrodes' contribution to the pain score by adding electrodes as covariates into the statistical model, Fz electrode was borderline, but not significantly associated with the subjective pain score ($p = 0.065$). The remaining nine electrodes showed no association with the subjective pain score ($p > 0.209$). A full statistical summary is shown in *Table 1*.

Table 1: Statistical summary of event-related synchronisation (ERS) in response to painful electrical stimuli. Values are EMM ± std err. ERS (%): the magnitude of change in oscillation amplitude is calculated as a percentage change in power for each time-frequency-point relative to a pre-stimulus baseline reference interval (- 900 ms to – 100 ms), displayed for "sleep", "expect" and "intens" conditions. * indicates a statistically significant change in oscillation magnitude at $p < 0.05$ level following FDR correction. There were no sleep x expect interactions or sleep x intens interactions at any electrodes (bottom left).

Event Related Synchronisation (ERS)

Electrodes	Sleep Condition								Expect Condition								Intens Condition			
	Habitual Sleep		Night Shift Work						Correct Signalling		Nocebo									
	Increase in activity (%)	Std err (%)	Increase in activity (%)	Std err (%)	dF	F value	p value	FDR - corrected	Increase in activity (%)	Std err (%)	Increase in activity (%)	Std err (%)	dF	F value	p value	FDR - corrected	dF	F value	p value	FDR - corrected
Fz	113.5	17.1	142.2	17.6	1 38	4.06	0.051	***p < 0.05**	134.1	16.4	121.6	16.1	1 242	2.94	0.088	p > 0.05	1 220	3.01	0.084	p > 0.05
Cz	140.6	18.7	167.9	19.3	1 35	2.86	0.100	p > 0.05	164.0	17.6	144.5	17.5	1 170	6.83	0.010	***p < 0.05**	1 220	2.96	0.087	p > 0.05
Pz	49.7	9.6	63.5	10.0	1 38	4.06	0.051	***p < 0.05**	58.9	9.5	54.3	9.4	1 236	0.98	0.322	p > 0.05	1 239	2.21	0.139	p > 0.05
C3/4c	99.1	14.1	119.8	14.5	1 38	3.87	0.057	***p < 0.05**	116.3	13.8	102.6	13.5	1 219	5.31	0.022	***p < 0.05**	1 201	4.09	0.045	p > 0.05
FC1/2c	124.3	18.0	152.9	18.4	1 34	3.50	0.070	***p < 0,05**	144.6	16.9	132.6	16.8	1 186	3.03	0.083	p > 0.05	1 183	2.48	0.117	p > 0.05
CP1/2c	85.1	12.4	100.0	12.8	1 209	5.12	0.025	***p < 0.05**	101.3	12.7	83.8	12.4	1 249	8.06	0.005	***p < 0,05**	1 239	0.69	0.406	p > 0.05
F3/4c	91.9	13.2	115.5	13.5	1 37	3.78	0.059	***p < 0,05**	108.1	12.3	99.3	12.2	1 247	2.17	0.142	p > 0.05	1 246	4.90	0.028	p > 0.05
P3/4c	41.9	8.0	56.2	8.5	1 267	7.49	0.007	***p < 0.05**	50.5	8.1	47.5	8.2	1 251	0.41	0.520	p > 0.05	1 251	1.69	0.195	p > 0.05
FC5/6c	88.0	11.9	97.0	12.2	1 40	0.70	0.407	p > 0.05	96.4	11.1	88.6	11.0	1 162	2.61	0.108	p > 0.05	1 234	5.11	0.025	p > 0.05
CP5/6c	62.4	10.0	76.1	10.6	1 42	3.30	0.077	***p < 0.05**	73.5	10.0	65.0	9.8	1 209	2.80	0.096	p > 0.05	1 210	3.86	0.051	p > 0.05

* $p < 0.05$ after FDR correction
Sleep x Expect condition $p > 0.182$
Sleep x Intens condition $p > 0.147$

ROI 2: Gamma Band Oscillations

There was no significant main effect of sleep condition ($p > 0.221$) or expect condition ($p > 0.335$) on GBO activity. There was a significant main effect of stimulus intensity and GBOs at CP1/2c electrode ($F(2, 179) = 5.36$, $p = 0.006$) and P3/4c electrode ($F(2, 151) = 7.91$, $p = 0.001$) [see *Table 2*]. Correcting for FDR did not alter the number of significant findings. There was no interaction between sleep and expect condition observed at any of the electrodes ($p > 0.239$). There was no interaction between sleep and intensity condition observed at any electrodes ($p > 0.052$). When assessing the individual electrodes' contribution to the pain score by adding electrodes as covariates into the statistical model, C3/4c and P3/4c electrodes were significantly associated with the subjective pain score ($p = 0.038$ and $p = 0.018$, respectively). Correcting for FDR did not alter the number of significant findings. A full statistical overview of GBOs is presented in *Table 2*.

Table 2: Statistical summary of gamma band oscillations (GBOs) in response to painful electrical stimuli. Values are EMM ± std err. GBOs (%): the magnitude of change in oscillation amplitude is calculated as a percentage change in power for each time-frequency-point relative to a pre-stimulus baseline reference interval (- 900 ms to – 100 ms), displayed for "sleep", "expect" and "intens" conditions. * indicates a statistically significant change in oscillation magnitude at $p < 0.05$ level following FDR correction. There were no sleep x expect interaction or sleep x intens interactions at any electrodes (bottom left). *PT:* pain threshold.

Gamma Band Oscillations (GBOs)																										
Electrodes	**Sleep Condition**								**Expect Condition**								**Intens Condition**									
	Habitual Sleep		Night Shift Work						Correct Signalling		Nocebo						2 X PT		3 X PT		4 X PT					
	Increase in activity (%)	Std err (%)	Increase in activity (%)	Std err (%)		dF	F value	p value	Increase in activity (%)	Std err (%)	Increase in activity (%)	Std err (%)		dF	F value	p value	Increase in activity (%)	Std err (%)	Increase in activity (%)	Std err (%)	Increase in activity (%)	Std err (%)		dF	F value	p value	FDR - Corrected
Cz	6.8	2.6	6.1	2.6	1	389	0.11	0.743	8.2	2.4	4.7	2.8	1	315	0.16	0.687	4.1	2.6	8.1	2.7	7.3	3.3	2	210	1.35	0.262	p > 0.05
Pz	5.4	2.0	7.5	2.2	1	40	0.71	0.404	6.6	1.7	6.3	2.1	1	213	0.93	0.335	5.0	1.9	8.0	2.0	6.4	2.3	2	162	1.25	0.289	p > 0.05
C3/4c	3.8	3.5	6.7	4.0	1	403	0.49	0.486	6.1	3.4	4.4	4.3	1	300	0.84	0.361	-0.3	3.8	5.9	4.0	10.2	5.3	2	206	2.02	0.136	p > 0.05
FC1/2c	9.1	3.3	5.5	3.3	1	405	1.50	0.221	8.6	3.1	6.0	3.5	1	49	0.37	0.545	6.5	3.3	6.0	3.3	9.4	4.4	2	201	0.32	0.729	p > 0.05
CP1/2c	7.9	2.2	6.6	2.4	1	44	0.23	0.632	8.1	1.9	6.4	2.3	1	286	0.02	0.894	3.3	2.1	10.9	2.2	7.6	2.8	2	179	5.36	0.006	***p < 0.05**
P3/4c	8.5	2.6	11.9	2.8	1	42	1.43	0.238	9.3	2.4	11.1	2.6	1	289	0.44	0.509	5.4	2.4	13.8	2.7	11.3	2.9	2	151	7.91	0.001	***p < 0.05**
CP5/6c	8.0	3.1	7.7	3.4	1	369	0.01	0.930	9.2	3.1	6.4	3.5	1	288	0.56	0.455	5.6	3.3	5.8	3.3	12.1	4.9	2	182	0.85	0.430	p > 0.05

* $p < 0.05$ after FDR correction

Sleep x Expect condition $p > 0.239$

Sleep x Intens condition $p > 0.052$

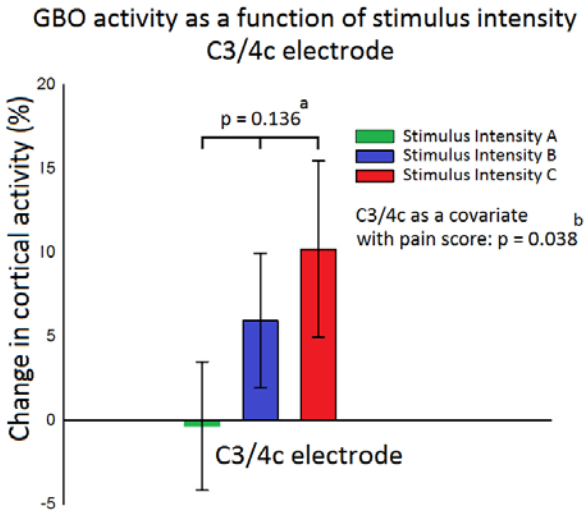

Figure 10: GBOs at C3/4c electrode as a function of stimulus intensity. The y-axis displays the change in cortical activity expressed as percentage change from baseline, (EMM ± std err). **a:** C3/4c electrode displays a step-like increase in magnitude in response to increasing stimulus intensity. This is however not significant. **b:** With the mean pain score as the dependant variable and C3/4c electrode was included in the statistical model as a covariate, C3/4c electrode was significantly associated with the subjective pain score.

ROI 3: α-Event-related desynchronisation and pre-stimulus α-level :

There was no main effect of sleep condition ($p > 0.240$) or expect condition ($p > 0.156$). There was a non-significant tendency for an effect of stimulus intensity observed at FC5/6c and C3/4c electrodes ($p = 0.050$ and $p = 0.057$, respectively). There was no interaction between sleep and expect condition ($p > 0.276$) or sleep and stimulus intensity condition ($p > 0.074$) observed at any electrodes.

A *post-hoc* analysis was performed investigating the difference between *pre-stimulus* and *pre-warning* α-level activity using a paired t test (see *Figure 3*). There were no differences observed at Fz, Cz and Pz electrode ($p = 0.502$, $p = 0.920$, and $p = 0.649$, respectively). There was however a significant difference observed at Oz electrode ($p = 0.017$).

Further post-hoc analysis using pre-warning showed that there was still no significant main effect of sleep condition ($p > 0.139$). There was however a significant main effect of expect condition observed at Fz electrode ($F(1,209) = 5.25$, $p = 0.023$) and P3/4c electrode ($F(1, 193) = 4.79$, $p = 0.030$), in which nocebo was associated with a reduced α-ERD compared to correctly signalled stimuli [see *Figure 11*]. After correcting for FDR, there were no significant findings on expect condition at $p < 0.05$ level. There was a significant main effect of stimulus

intensity observed at Pz electrode (F (1, 227) = 4.48, p = 0.035), however, the remaining electrodes did not show a significant effect of stimulus intensity (p > 0. 185). There was no interaction between sleep and expect condition (p > 0.328). There was a significant interaction between sleep and stimulus intensity at CP5/6c electrode (F (1, 245) = 4.85, p = 0.029), however, the remaining electrodes did not show a significant interaction between sleep and stimulus intensity (p > 0.107).

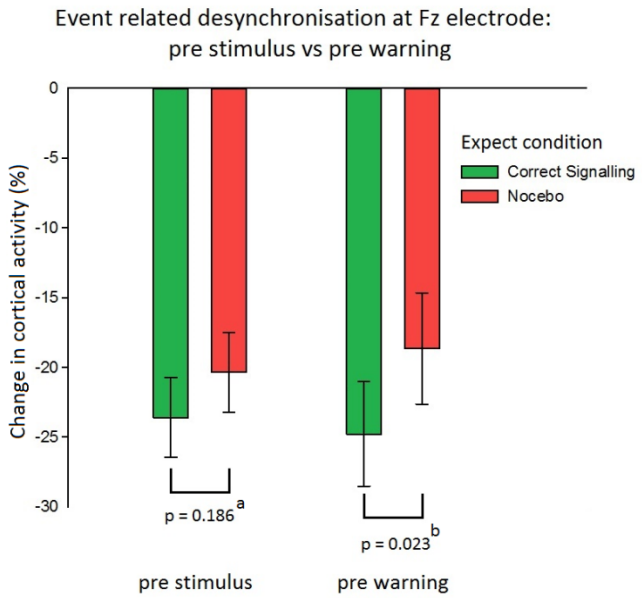

Figure 11: Difference in ERD at Fz electrode in response to painful electrical stimuli. The y-axis represents percentage change in cortical activity (EMM, ± std err). **a:** Using pre-stimulus as baseline measure, there is no significant effect of expect condition on α-ERD. **b:** Using pre-warning as baseline measure displays a significant effect of expect condition, in which nocebo leads to a significantly reduced α-ERD compared to correctly signalled stimuli.

A paired t-test was performed to evaluate the effect of sleep condition on pre-stimulus α-power on Cz electrode (F (1, 41) = 1.19, p = 0.281) and C3/4c electrode (F (1, 40) = 0.22, p = 0.641). Similarly, the same investigation was performed for the effect of sleep condition on pre-warning data on α-power at Cz electrode (F (1, 33) = 0.85, p = 0.364) and C3/4c electrode (F (1, 36) = 0.01, p = 0.912), demonstrating that the pre-stimulus/pre-warning α-power remained stable across the experiments.

Discussion

This study has shown that following two nights of NSW, the participants exhibited hyperalgesia in response to painful electrical stimuli, which was accompanied by specific time-frequency responses. Moreover, the present study extends previous experimental knowledge of SIH into clinical findings in a cohort of nurses working night shifts. Following NSW the participants were sleepier, as indicated by the KSS and PVT scores. These measures confirm that the participants experienced sleep deprivation effects from working night shift and allows for comparison with other studies.

Pain perception:

Following NSW the participants exhibited SIH in response to painful electrical stimuli. The participants reported an increase in pain of ≈ 22% following NSW, demonstrating that the protocol successfully managed to study nurses that were in deed experiencing SIH [see Figure 5]. Previous experimental studies report SIH in response to laser-induced pain following partial sleep restriction [SR] (Tiede et al., 2010) and total sleep deprivation [TSD] (Azevedo et al., 2011, Schuh-Hofer et al., 2015). Additionally, Schuh-Hofer et al. (2013) report reduced pain threshold to heat, cold and mechanical pinpricks and cold hyperalgesia in response to one night of TSD. Recently, Matre et al. (2015) reported SIH in response to painful electrical stimuli and increased pressure pain sensitivity in healthy volunteers exposed to partial SR. To the author's knowledge, the present study is the first to demonstrate SIH in a cohort of nurses working night shifts. This lends supports to previous studies reporting an association between working night shifts and increased self-reported pain complaints (Zhao et al., 2012, Buja et al., 2013, Barro et al., 2015, Takahashi et al., 2015).

The present study reports a comparatively smaller percentage increase in pain scores compared to other studies investigating SIH. Tiede et al. (2010) and Schuh-Hofer et al. (2015) reported a 30% and 37% increase in pain scores following laser-induced heat pain, respectively. One possible explanation for the observed difference could be due to methodological differences: Laser-induced heat pain is known to stimulate Aδ and C-fibres (Bromm and Treede, 1984), whereas electrical pain stimulation reportedly also activates Aβ-fibres (Baumgartner et al., 2012). Although speculative, it may be that the activation of the

Aβ-fibres actually leads to a gating of the nociceptive transmission at the dorsal horn, in line with the pain-gate theory proposed by Melzack and Wall (1965).

The differences between the present study and the results from Matre et al. (2015) which both use electrical pain stimulation, may reflect subtle differences in total amount of sleep. Matre et al. (2015) reported approximately 8% increase in pain ratings following SR. The volunteers in the study by Matre et al. (2015) slept 50% less for two consecutive nights and were thus partially sleep deprived. As the present study included nurses on an actual clinical shift rota, it is likely that at the nurses were more sleep deprived than the volunteers from Matre et al. (2015). Consequently, it is likely that they experienced a SIH closer to that reported from experimental studies using TSD (Schuh-Hofer et al., 2013, Schuh-Hofer et al., 2015).

The subjective pain scores are however, not comparable to the results reported by Azevedo et al. (2011), which reported a 57% increase in subjective pain scores following 48 hours of TSD. Although the present study investigated nurses following two nightshifts, the participants were allowed to sleep during the day between the consecutive nightshifts, which may have reduced the subsequent hyperalgesia. This is in line with a recent study by Faraut et al. (2015) in which 30 minutes of daytime napping twice a day reversed the hyperalgesic effects of SR. It was beyond the scope of this thesis to investigate whether there was any correlation between the subjective pain score and amount of sleep reported by the nurses. It would however, serve as an important topic to investigate in future studies, as napping could have an important role in preventing the development of pain in people exposed to sleep restrictions through work.

Nocebo

The present study also investigated the effect of negative expectations (nocebo) on painful electrical stimuli. The participants reported a nocebo-induced hyperalgesia (NIH) of ≈ 20%, demonstrating that the nocebo procedure was correctly understood and remembered across sessions by the participants. According to a recent meta-analysis investigating the magnitude of nocebo in pain, the nocebo effect is moderate to large, but highly variable with verbal warnings provided alongside conditioning procedures yielding the largest nocebo effect (Petersen et al., 2014). The level of NIH in the present study is higher than that reported by Lorenz et al. (2005), in which low intensity laser stimuli cued as high intensity

stimuli were significantly more painful than correctly cued low intensity stimuli. They report an average increase of pain score of approximately 8-10%. However, it is difficult to make direct comparison with the present study, as Lorenz et al. (2005) relied on a 9 point scale ranging from 0-8 and used laser heat as test stimulus.

The NIH from the present study is less than that reported by Colloca et al. (2010), who investigated the nocebo response to electrical painful stimuli cued by red, yellow or green lights. They report a NIH of ≈50%, however, the research protocol used by Colloca et al. (2010) utilised non-painful and painful signals, which might yield greater differences compared to the present study, which relies on three different painful stimuli. Moreover, they maintain that nocebo is less reliant on learning mechanisms compared to placebo. This latter finding is supported by the current study, as the research protocol did not rely on extensive training prior to the actual experiments. Thus, the level of NIH reported from the present study is in the middle of the lower and higher levels reported in the literature, adding further support to Petersen et al. (2014) who report large variations in nocebo magnitude.

Although the nocebo pain scores were increased following NSW, there was no sleep x expect interaction ($p = 0.438$), indicating that the SIH is not explained by alterations in negative expectations [see *Figure 6*]. This is noteworthy, considering that sleep restriction is associated with negative mood changes (Haack and Mullington, 2005, Simon et al., 2015), which may potentially lead to increased pain sensitivity. A potential mechanism highlighting the link between sleep and expectations is elaborated on below.

Laverdure-Dupont et al. (2009) investigated the effect of sleep stages and the placebo response. Healthy volunteers were introduced to a placebo and measured for a placebo response following a daytime delay of 12 hours or an overnight delay of 12 hours. There was no placebo response following the daytime delay, however, there was a placebo response following the overnight delay. Interestingly, the level of placebo-analgesia was related to the amount of REM sleep, which was measured by polysomnography. The authors propose that reduced REM sleep is associated with a facilitation of expectancy-mediated responses, arguing that the mechanisms may be related to sleep-induced learning processes (Laverdure-Dupont et al., 2009). Consequently, if one were to extend the findings from

Lavedure and colleagues to the present study and nocebo, we would expect to see a strengthened connection between negative expectations (nocebo) and hyperalgesia, due to the lack of REM sleep following NSW. The lack of such findings could potentially stem from subtle differences in responses from the participants. Laverdure-Dupont et al. (2009) divided the responders into placebo-responders and non-responders and a similar procedure of nocebo-responders and no-responders in the present study might have disclosed a subgroup of nocebo-responders whose SIH is reflected by negative expectations.

Alternatively, it is argued by Benedetti (2013) that there are many placebo effects and thus, arguably many nocebo effects. Consequently, it may be that a nocebo procedure relying on other mechanisms, such as anxiety and reward, could possibly have demonstrated a closer associations between nocebo and SIH. However, it is argued by Colloca et al. (2008) and Colloca et al. (2010) that learning does not influence the nocebo response and Colagiuri et al. (2015) maintain that nocebo procedures lead to heightened anxiety and seem resistant to extinction, irrespective of the nocebo procedure. Thus, in the present study, it is in the author's opinion unlikely that a nocebo procedure utilising other mechanisms, such as fear, would establish a causal link between SIH and NIH.

Taken together, the present study confirms existing knowledge regarding electrical painful stimuli and SIH and extends that into a clinical cohort of nurses working night shifts. SIH is present in a cohort of nurses following two nightshifts and thus, the main hypothesis regarding SIH is supported. Regarding NIH, there is support for the hypothesis that nocebo leads to hyperalgesia, however, there does not seem to be support for the hypothesis regarding nocebo as one of the underlying mechanisms explaining SIH. This may partly be due to methodological matters, as different nocebo procedures may act upon on various underlying mechanisms. Nevertheless, the present study does not find support for negative expectations as a principal underlying factor in SIH.

ROI 1: Event-related synchronisation

Sleep Condition:

The TFA demonstrated an ERS across all electrodes following NSW compared to HS and was statistically significant at 8/10 electrodes ($p < 0.05$) [see *Table 1*]. To the author's knowledge, this is the first study to report a sleep-induced facilitation of ERS across several electrodes in response to painful electrical stimuli in a cohort of nurses working nightshift. The ERS was

evident in the 1-400 ms/1-25 Hz, post stimulus interval. This is similar to the findings reported by Matre et al. (2015) who investigated the TF responses to electrical pain stimuli in healthy volunteers after two nights of partial SR.

There are however, some differences between the two studies, most notably the magnitude of the ERS. Matre et al. (2015) found a significant effect of SR at Cc electrode (C3/4c), in which the magnitude of the ERS was 85% and 108% larger than baseline for HS and SR, respectively. The equivalent numbers from the present study at C3/4c electrode were 99% and 120% for HS and NSW, respectively. Thus, it seems that the present study reports a slightly larger increase in magnitude following painful electrical stimuli compared to that reported by Matre et al. (2015). As noted previously, this could reflect differences in degrees of sleep deprivation, in which the participants in the present study are most likely experiencing TSD, as opposed to partial SR. However, the percentage increase from HS to NSW/SR is similar (≈ 22-23% increase) in both studies at C3/4c electrode. Given that the increase in subjective pain ratings are not comparable (≈ 8% vs ≈ 22%), but the ERS is, it is possible that the ERS observed at the C3/4c electrode represents an objective phenomenon of sleep deprivation that is worth pursuing. There are however, no other studies reporting TFA following sleep deprivation, although possible explanations may be postulated, which is elaborated on below:

According to Gram et al. (2015), activity in the theta bandwidth (4-8 Hz) is highly associated with pain perception. Gram and colleagues investigated the responses of 39 participants exposed to a cold pressor (CP) test on two days, separated by 7 days, and compared the subjective pain ratings to the corresponding cortical activity between 1-70 Hz. Although several bandwidths (theta, beta and gamma) showed a correlation to the pain score, the theta bandwidth was reportedly the most dynamic and reliable indicator of pain perception. The results are not directly comparable to the present study, as Gram et al. (2015) used a tonic, experimental painful stimulus, by way of the CP test, whereas the present study relies on electrical painful stimuli. Additionally, the cluster of activity that constitutes the central core of ROI 1, spans over a greater bandwidth (1-12 Hz) than that Gram et al. (2015) used. Statistically however, when investigating the subjective pain score as the dependant variable and the individual electrodes were included as covariates, Fz electrode ROI 1 activity showed a non-significant tendency for explaining the subjective pain score ($p = 0.065$). Thus, in line

with the reasoning proposed by Gram et al. (2015), increased theta activity may potentially be an interesting objective marker of sleep-induced hyperalgesia, which warrants further investigation. This is further supported by Schulz et al. (2011) who found greatest inter-individual consistency to laser-evoked pain responses in the theta bandwidth.

Alternatively, it may be that the increase in magnitude following NSW represents a global cortical phenomenon, in which the brain responds to a salient stimuli more intensively following sleep restrictions. The saliency of a stimulus has been defined as the ability to stand out from other sensory stimuli and nociceptive stimuli seem ideally suited for that purpose (Chien et al., 2014). Moreover, Mouraux and Iannetti (2009) argue that the saliency of a stimulus is reflected in the actual intensity of the stimulus. Furthermore, Tiemann et al. (2015) found a significant increase in cortical magnitude in the theta bandwidth (4-8 Hz) between 150-350 ms post stimulus in response to painful laser stimuli. The authors investigated the pooled, averaged response from central electrodes, (FCz, Cz and C2) and report an increased activity in response to increasing intensity. The results from the present study show a similar trend, with Fz, Cz and C3/4c electrodes' magnitude associated with stimulus intensity ($p = 0.084$, 0.087 & 0.045, respectively). However, as Tiemann et al. (2015) used laser and investigated the average responses from a group of electrodes, results are not entirely comparable.

Thus, the present study extends experimental findings from Matre et al. (2015) and reports increased ERS in the 1-400 ms/1-25 Hz, post stimulus interval in nurses working nightshift. Our hypothesis regarding an increased ERS magnitude following NSW is thus supported.

Expectation condition (nocebo):

The nocebo procedure consistently produced a smaller increase in cortical power compared to correctly signalled stimuli across all electrodes and was significant at Cz, C3/4c and CP1/2c electrodes ($p < 0.05$). To the author's knowledge, this is the first study to report time-frequency specific changes observed with a nocebo procedure following sleep restrictions. The most plausible explanation for the observed reduction in ERS following nocebo compared to correctly signalled stimuli, is that the saliency of a stimulus is reflected in the intensity of the stimulus (Mouraux and Iannetti, 2009). This has recently been supported by Tiemann et al. (2015) and discussed previously. Thus, nocebo stimuli, although perceived as

more painful, do not seem to capture more attention than the actual intensity of the signals warrant.

Tiemann et al. (2015) further argue that under circumstances where pain perception is dominated by salience-detection and affective processes, operculoinsular and cingulate cortices display non-specific pain related activity, in line with that proposed by Legrain et al. (2011). The results from the present study seem to support the notion that cingulate cortices are involved in expectancy-mediated processes. Electrodes Cz, C3/4c and CP1/2c overlying the fronto-central cingulate cortices are significantly associated with nocebo ($p < 0.022$), whereas F3/4c, P3/4c and FC5/6c electrodes are not ($p > 0.108$).

This is different to that reported by Lorenz et al. (2005) who used a combination of EEG and MEG registration in response to laser evoked potentials (LEPs) and nocebo and placebo procedures. Using source-localisation, they found that activity in the SII was highly correlated with pain intensity and effect of expectations (placebo and nocebo), whereas the cingulate cortices only showed an association with stimulus intensity. It is however, difficult to compare the findings from the present study to those reported by Lorenz et al. (2005) for several reasons: For one, source-localisation relies on a-priori assumptions and may therefore bias the results (Hu et al., 2013). More importantly, there are methodological differences, such as time-domain vs time-frequency and laser vs electrical pain stimulus. Additionally, Lorenz et al. (2005) have a skewed distribution of the warning signal: 80% were correctly signalled whereas the remaining 20% were erroneously signalled. Consequently, the findings from Tiemann et al. (2015) and the present study indicate that expectations (placebo-nocebo) related to pain processing may be reflected in activity in electrodes overlying the fronto-central cingulate cortices.

Finally, the disparity between the subjective pain score and cortical activity is worth exploring. As noted previously, in the present study the pain score is consistently higher following nocebo and mirrors the SIH, whereas the ERS magnitude is consistently lower following nocebo, compared to correctly signalled stimuli [see *Figure 9*]. According to Schulz et al. (2011), time-frequency responses in the lower frequency range (theta, 3-8 Hz) correspond to time-domain evoked potentials and reflect changes in bottom-up processing. Results from studies using conventional time-domain analyses indicate that there is

increased pain perception accompanied by a reduction of the amplitude of the pain evoked potential in sleep deprived subjects (Tiede et al., 2010, Schuh-Hofer et al., 2015). Additionally, it is proposed by Ødegard et al. (2015) that this may be due to reduced attentional reorientation towards painful stimuli with subsequent increased perceptual amplification. Thus, the similarity between the SIH and NIH reflected in the subjective pain scores and the disparity between the ERS magnitude in nocebo and sleep evoked potentials, seem to support the notion that the SIH and NIH are not explained by bottom up mechanisms. Consequently, it appears that the perceptual amplification most likely responsible for the hyperalgesia observed in SIH and NIH stem from perceptual processes that do not overlap. As such, whereas the SIH may to a certain extent be reflected in the ERS in the theta bandwidth, the cortical network activity responsible for nocebo does not seem to be reflected adequately in the 1-400 ms/1-25 Hz, post stimulus interval. Thus, it seems that although the SIH and NIH are comparable in subjective pain scores, the two phenomena are not represented by similar cortical network activity pattern.

Taken together, the presents study reports an increase in cortical activity in the delta-theta bandwidth following NSW and there is support for the hypothesis regarding frequency-specific changes in SIH. However, although nocebo consistently lead to reduced cortical activity compared to correctly signalled stimuli, the observed NIH does not seem to be explained by specific time-frequency cortical activity patterns in the in the 1-400 ms/1-25 Hz, post stimulus interval.

ROI 2: Gamma Band Oscillations

The TFA displayed a significant cluster of activity in the gamma frequency range (GBOs) in the 100-200 ms post stimulus interval in seven out of ten electrodes [see *Figure 4, & Table 2*]. At electrodes Fz, F3/4c and FC5/6c the bootstrapping procedure did not identify significant clusters of activity and are therefore not included in the discussion.

Sleep condition:

At the seven electrodes in which GBOs were identified, none showed an effect of sleep condition or nocebo. This is in accordance with previous findings (Matre et al., 2015) who reported that the GBOs did not change with experimental sleep restrictions. Together, these findings indicate that GBOs do not reflect the hyperalgesia observed following sleep-restrictions.

This is noteworthy, given that several studies point to GBOs and their potentially important role in encoding of pain intensity (Zhang et al., 2012, Schulz et al., 2011) and attentional modulation of pain processing (Hauck et al., 2007a). Recently, Hauck et al. (2015) using TFA and source-localisation argued that GBOs are sensitive to both bottom up (stimulus intensity) and top down (attention) modulation of experimental laser-induced pain. They also reported that activity in the cingulate gyrus (CG) and SII are consistently activated by pain and proposed that GBOs observed at the CG and SII reflect the activity of a network involved in the multidimensional integration of pain. Interestingly, Tiemann et al. (2015) report that gamma responses are sensitive to changes in stimulus intensity but not to placebo and argue that GBOs reflect sensory processing of nociceptive signals at SI level. They further propose that GBOs are sensitive to sensory discriminative aspects of pain, but not necessarily the affective and evaluative components of pain perception.

The findings from the present study seem to support the view advocated by Tiemann et al. (2015). There was a significant effect of stimulus intensity at CP1/2c and P3/4c electrodes and although not statistically significant ($p = 0.136$), C3/4c electrode showed a "step-like" increase in activity in response to increasing stimulus intensity (see *Figure 10*). Additionally, when assessing the individual electrodes' contribution to the pain score by adding electrodes as covariates into the statistical model, C3/4c was significantly associated with the subjective pain score ($p = 0.038$). However, as there was no effect of nocebo on GBOs activity, the findings from this study indicate that GBOs are involved in the sensory processing of painful electrical stimuli, but do not reflect the complicated integration of pain perception previously reported by Hauck et al. (2015) or indeed, the processes underlying SIH.

ROI 3: α-event-related desynchronization (α-ERD)

Sleep condition:

Previous studies report a global α-ERD following the exposure to painful stimuli (Ohara et al., 2004, Iannetti et al., 2008). The results from the present study indicate that α-ERD is not a phenomenon unquestioningly associated with painful stimuli. Recently, Matre et al (2015) reported a reduction of α-ERD observed at Cc (C3/4c) electrode following sleep restriction. The authors speculate as to whether this could be explained by a reduced sensory-discriminative processing in the somatosensory cortex, leading to an increased affective

processing of the nociceptive stimuli and hence, increased pain perception. However, the results from the present study seem to oppose this viewpoint, as the effect of NSW at C3/4c electrode was not significant ($p = 0.368$). Indeed, in the present study, the effect of NSW on cortical magnitude was not reflected in the α-oscillations at any electrodes. A direct comparison between the two studies is hampered by methodological differences. In the study by (Matre et al., 2015), the participants were not exposed to a nocebo and consequently, they may exhibit a more distinct effect of sleep restrictions. The results from the present study are drawn from data containing a nocebo which could potentially offset the analysis. A possible explanation is provided by Jensen and Mazaheri (2010).

Jensen and Mazaheri (2010) propose that alpha activity is related to the engagement or disengagement of specific brain regions. Briefly, the authors argue that information is gated through the brain by "functional inhibition", in which task-irrelevant areas of the cortex are inhibited by alpha activity. Specifically, they authors argue that alpha activity decreases in engaged areas and increases in disengaged areas and this inhibition allows for communication between regions in the gamma frequency band (Jensen and Mazaheri, 2010). Thus, although speculative, it may be that the introduction of a nocebo in the present study led to a different "gating" of the painful stimuli in the alpha bandwidth compared to that reported by Matre et al. (2015). As such, it would be of interest to compare the alpha activity with subsequent gamma activity in future studies. However, as the results were analysed using both pre-stimulus and pre-warning as baseline references, it is in the author's opinion unlikely that the lack of sleep-induced α-ERD in response to painful electrical stimuli is due to methodological matters.

The role of alpha oscillations in pain perception is also disputed by the findings of Schulz et al. (2011) who investigated pain perception using linear mixed models as a statistical means. The authors report that whereas theta and gamma activity improved the statistical model in explaining the individual pain perception, the alpha responses did not. They further propose that alpha activity merely echoes the preceding theta and gamma activity (Schulz et al., 2011). Contrary to these findings, Babiloni et al. (2006) report that the strength of the anticipatory α-ERD is highly associated with the subsequent subjective pain ratings. Briefly, the authors report that a strong anticipatory α-ERD was indicative of a higher subjective pain score, and this was particularly evident at the electrodes overlying the SI. The authors

propose that the anticipatory α-ERD reflects cortical processes related to the conscious evaluation of pain intensity (Babiloni et al., 2006). It was beyond the scope of this thesis to investigate the extent to which anticipatory α-ERD affected the subjective pain scores, however, the disparity between the psychophysical and neurophysiological measures regarding the α-oscillations and pain perception warrants further investigations.

Expectation condition (nocebo):

Recently, Hu et al (2013) investigated α-oscillations in response to nociceptive electrical stimuli and the difference between exogenous sensory-related and endogenous task-related activity. They report that the sensory-related α-ERD was mostly reflected at the contralateral somatosensory cortex and the endogenous task-induced α-ERD was most strongly reflected at the posterior parietal and occipital cortices. The present study lends some support for this hypothesis, as there was a borderline significant effect of stimulus intensity observed at C3/4c electrode ($p = 0.057$), probably reflecting the exogenous sensory-related α-ERD overlying the somatosensory cortex. The findings from the occipital electrodes (Oz and O1/2c) however, do not support the task-induced α-ERD reported by Hu et al (2013), with p values at 0.467 and 0.156, respectively. One potential mechanism for this is outlined below:

As cognitive tasks are associated with α-ERD (Lopes da Silva, 2013), the data were analysed using pre-warning as a secondary reference point. Changing from pre-stimulus to pre-warning did not change the results regarding the effect of NSW or stimulus intensity on α-ERD activity level. It did however, seem to affect the subsequent nocebo analysis [see *Figure 11*]. Notably, using pre-warning as baseline measurement displayed a significant effect of nocebo on activity at the Fz and P3/4c electrodes ($p = 0.023$ and 0.030, respectively). This lends support to the opinion of Hu and colleagues in that cognitive tasks lead to a task-induced α-ERD. However, the activity from the present study is topographically somewhat different, reporting α-ERD at Fz electrode in addition to the activity at the posterior parietal and occipital cortices reported by Hu et al (2013). Recently, Hauck et al. (2015) reported that α-ERD was modulated by both attention and stimulus intensity and was most pronounced over the central electrodes adjacent to Cz.

Potential reasons for these differences could be due to methodological differences. Hu et al did not include sleep restrictions in their study design and they also divided the α-oscillations into early (250-350 ms) and late (400-750 ms) post stimulus intervals. Additionally, Babiloni

et al. (2014) report that the alpha bandwidth may be subdivided into high and low frequencies (10-12 Hz and 8-10 Hz, respectively) and that these subcategories have separate functional tasks in sensory and nociceptive processing. In the present study and those of Hu et al (2013) and Hauck et al. (2015), the alpha bandwidth is not subcategorised into high and low alpha and consequently, direct comparison between studies is hampered.

Consequently, some of the findings from the present study are in line with previous findings and the hypothesis regarding α-ERD and responses to noxious stimuli is partially accepted. The implication from the present study is that future studies investigating the effects of expectations may potentially benefit from using a pre-stimulus baseline which is based on cortical activity prior to the warning signal, in order to reduce the possibility of a floor effect of the subsequent post-stimulus measurements. Additionally, sub-categorising the alpha oscillations into high and low alpha oscillations, as proposed by Babiloni et al. (2014) may yield more consistent findings across studies. However, the present study does not find support for specific sleep-induced alterations in cortical magnitude expressed as α-ERD.

Limitations

There are several issues that need to be addressed regarding the present study. The first one pertains to the methodology. TFA investigates the parameters that control oscillations in neuronal networks (Pfurtscheller and Lopes da Silva, 1999), however, surface EEG recordings will mostly reflect sub-cranial activity (Luck, 2014). Given that pain perception is a multifaceted phenomenon, scalp-EEG recordings will not be able to sufficiently address the deeper cortical structures that are involved in pain perception, such as the hippocampus and the basal ganglia. Likewise, brain recordings which are broken down into separate pre-specified time intervals, may not fully comprehend the constant flow of cortical information that ultimately culminate in pain perception. The use of LMM allows for more specific and individualised analysis of the data, allowing for within-subjects analysis and single trials inclusion (Vossen et al., 2011). The within-subjects component seems particularly relevant, as it enables the study of individual differences across a diverse phenomenon, such as pain perception.

Then there is the confounding issue of pain as a complex, multifactorial phenomenon, which cannot under any circumstances be reduced to a point score on a VAS. Moreover, there is a high degree of inter-individual variability in pain threshold and pain scores (Nielsen et al.,

2009). Consequently, a more comprehensive individual pain assessment, for instance the McGill pain questionnaire (Melzack, 1975), would have complemented the psychophysical and neurophysiological findings. Additionally, it may have proven valuable in assessing the effects of nocebo on SIH. Realistically though, the questionnaire may take as long as 30 minutes to complete, which would compromise the practicability of the experiment. Additionally, the main aim of the study was to investigate SIH and the neurophysiological responses to experimental, nociceptive-driven pain. Thus, additional psychophysical measurements would not have altered the neurophysiological responses and the conclusions drawn from the EEG analysis.

Another important issue is the use of warning signals. The intensity of the impending painful stimulus was indicated by a square, a circle and a triangle. There is a risk that the meaning of the warning signals was not remembered across sessions and that the warning signals may potentially be misinterpreted by the participants. Likewise, given the societal denotation to triangles and impending danger, there is a risk of a systematic bias, in which the triangle is remembered, but the circle and square may lead to mixed responses from the participants. However, the pain scores displayed a step-like increase for stimulus intensity A, B and C, and if the participants were uncertain regarding the impending stimulus intensity, it is likely that the correctly indicated signals would have been affected as well. Nevertheless, future studies investigating nocebo would probably benefit from using two different intensities, as opposed to three.

The present study investigated the effects of NSW on pain perception in a cohort of nurses and extends experimental findings into clinically relevant knowledge. It may not however, be representative for all types of night shift work. Indeed, there may even be differences within the same profession: A nurse working on a quiet ward may have opportunities for small naps during the night, whereas a nurse working on a busy intensive care unit may not. Consequently, future studies need to investigate whether the SIH observed in the participants in the present study is representative for other professions whose jobs involve night shifts.

Lastly, although the present study confirms the existence of SIH, experimentally induced pain responses do not mimic the suffering associated with chronic, disabling pain.

Nevertheless, experimental research may yield additional knowledge that may ultimately culminate in better prophylactic interventions for people at risk of developing pain and improved treatment options for people already suffering from chronic pain.

Conclusion

The present study extends previous experimental studies into clinical findings in a cohort of nurses working nightshifts. Following two nights of NSW, the participants exhibited hyperalgesia in response to painful electrical stimuli as measured by a VAS, which was accompanied by specific time-frequency responses. Specifically, the subjective hyperalgesia was reflected by pain-induced ERS in the 1-400 ms/1-25 Hz, post stimulus interval. Following NSW, there was a statistically significant increase in ERS at 8/10 electrodes compared to after HS. Consequently, ERS in the delta-theta bandwidth appears to be a consistent marker of SIH, however, the extent to which it may explain the complicated processes underlying pain perception remains uncertain. Contrary to this, GBOs and α-ERD do not seem to be objective neurophysiological correlates of SIH. However, GBOs in the 100-200 ms and ERS in the 1-400 ms post stimulus interval may represent global cortical phenomena, in which the brain responds to a salient stimulus more intensively following sleep restrictions.

Lastly, this study demonstrates that following a nocebo procedure, the participants demonstrated an increase in subjective pain score which was accompanied by a smaller ERS compared to correctly signalled stimuli. However, although NSW and nocebo lead to comparable increases in subjective pain scores, the objective neurophysiological cortical activity responsible for these perceptual amplifications do not seem to share the same cortical mechanisms. As such, the present study does not find support for negative expectations as a principal underlying factor in SIH, but rather, SIH and NIH appear to stem from cortical processes that do not overlap.

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Appendix 1: Recruitment poster

Statens arbeidsmiljøinstitutt

Forsøkspersoner søkes

Friske sykepleiere (18 - 60 år) som jobber 3-delt turnus, evt nattevakter, søkes til å delta i et forskningsprosjekt som undersøker fysiologiske effekter av skiftarbeid og lite søvn.

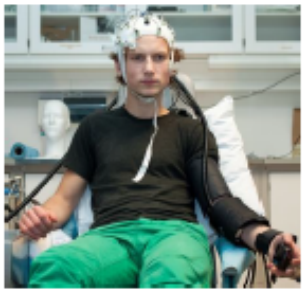
Deltakerne vil motta moderat smertefulle stimuleringer på huden. Denne type stimulering er alminnelig brukt i forskning. Elektrisk aktivitet fra hjernen (EEG) vil registreres samtidig.

Undersøkelsen strekker seg over i alt 7-8 timer fordelt på 3 ulike dager.

Deltakelse honoreres.

Forsøket gjennomføres i regi av Statens arbeidsmiljøinstitutt på Majorstua (Gydas vei 8) i Oslo.

Kontakt Statens arbeidsmiljøinstitutt for mer informasjon på tlf 40 72 17 88 eller e-post: forsok@stami.no.



Godtgjørelse: 150 kr/time (ca. 1.000 kr) + reiseutgifter. Godtgjørelsen er skattefri t.o.m 1.000 kr. Reiseutgifter med offentlig transport dekkes i stor-Oslo t.o.m Ruters sone 4.

Appendix 2: 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.

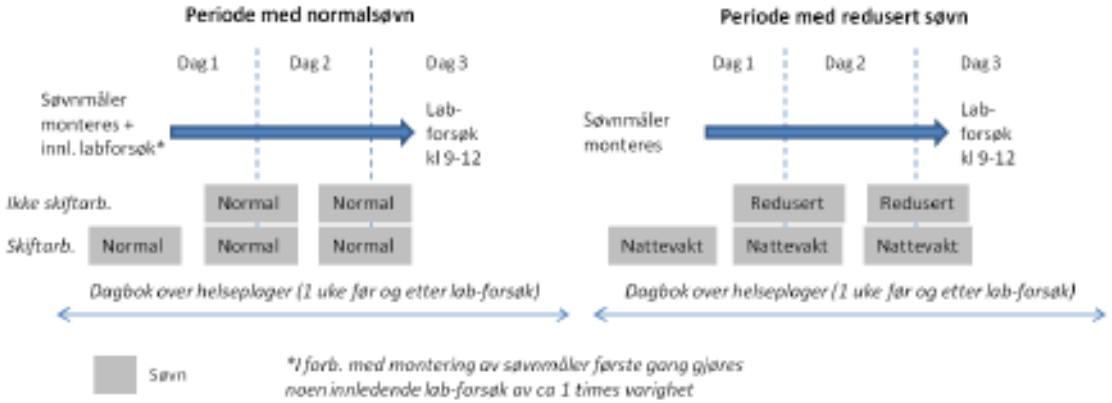
Del	Test[1]	Beskrivelse
1	Smerteterskler • Trykk • Varme • Kulde • Elektrisk	Smerteterskler bestemmes ved at ved at intensiteten på stimuleringen gradvis økes inntil moderat smerte kjennes og testen avbrytes. Gjentas 2-3 ganger for hver type stimulering.
	EEG monteres	En hette med 32 elektroder plasseres 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 3: Karolinska Sleepiness Scale

Hvor søvnig føler du deg nå?

Besvar spørsmålene ved å angi et tall Anvend gjerne mellomnivåene 2,4,6,8 også

1 veldig opplagt
2
3 opplagt
4
5 verken opplagt eller søvnig
6
7 søvnig, men ikke anstrengende å være våken
8
9 veldig søvnig, kamp mot søvnen, anstrengende å være våken