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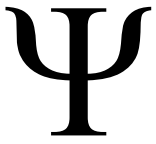

Effect of Aversive Stimuli on Frontal Alpha Asymmetry During Sleep

HOVEDOPPGAVE

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CONTENTS

Abstract

The aim of this project was to find measurable physiological responses linked to the human experience of distress in a non-communicative state. A distress-related response has consistently been reported in healthy awake subjects in the form of power spectrum alpha band changes in frontal brain areas. Since sleep is a state of sensory detachment it was used as a model to study physiologic distress responses in a non communicative state. Changes in frontal alpha asymmetry were observed in 27 volunteers exposed to aversive and neutral (control) stimuli during sleep. Power spectrum data from frontal electroencephalographic (EEG) leads (F3 / F4) was assessed in 2 second epochs before and after administration of pain and aversive conditioned stimuli during sleep. Additionally, changes in sleep quality were evaluated following presentation of aversive stimuli in sleep.

Results show that frontal alpha asymmetry changes (left > right) after aversive compared to neutral stimuli administration. Sleep was not significantly affected. Aversive stimulation produces measurable similar changes regardless of state (stage 2, rapid eye movement (REM) sleep, or wakefulness) in frontal alpha activity consistent with the implicit processing of negative emotions. This suggests that a measurable aversive response may possibly also be obtained in non-communicative states like dementia, anesthesia or coma during acute distress, providing an important clinical application

Sammendrag

Dette prosjektet søkte å finne målbare fysiologiske responser knyttet til opplevelsen av lidelse i ikke- kommuniserende tilstander. Slike responser knyttet til ubehag har blitt rapportert i friske, våkne forsøkspersoner som endringer i alfa bånd power spectrum data i frontale områder. Da søvn er en tilstand karakterisert av sensorisk inhibering ble tilstanden benyttet som eksperimentell modell til å studere fysiologiske korrelater til lidelse i en ikke- kommuniserende tilstand. Endringer i frontal alfa asymmetri ble observert i 27 forsøkspersoner som ble eksponert for ubehagelige og nøytrale stimuli under søvn. Power spectrum data fra frontal elektroencefalografiske (EEG) avledninger (F3/F4) ble analysert i 2 sekunders epoker før og etter administrering av smerte samt ubehagelige betingede stimuli under søvn. I tillegg ble det evaluert endringer i søvnkvalitet etter presentasjon av ubehagelig stimuli.

Resultatene viser at frontal alfa asymmetri endres (Venstre > Høyre) etter administrering av ubehagelig, i forhold til nøytralt stimuli. En fant ikke signifikante endringer i søvnkvalitet. Ubehagelige stimuli førte til like og målbare endringer i alfa asymmetri tilsvarende implisitt prosessering av negative emosjoner, uavhengig tilstand (S2, REM søvn eller våkenhet). Dette indikerer at en respons under akutt lidelse formodentlig kan måles også i ikke- kommunikative tilstander som demens, anestesi, eller koma, noe som vil kunne representere et viktig klinisk instrument.

Introduction

Aim

This study aimed to find measurable physiological responses linked to the human experience of distress in conditions where no overt communication is possible. Such conditions are usually pharmacologically induced (e.g. anesthesia) or pathological (e.g. dementia and coma). It has been suggested that distress in these circumstances may produce a strong negative emotional experience (like feelings of hopelessness, suffering, agony and misery) that goes largely unnoticed (Magarey & McCutcheon, 2005; Orser, Mazer, & Baker, 2007; Sandin, Enlund, Samuelsson, & Lennmarken, 2000).

In this project, conditioned aversive and painful stimuli were administered in S2 and REM sleep. The intention was to generate comparable types of non-communicable negative emotional responses, assessed as changes in frontal alpha power. In addition, it was considered if the presentation of conditioned or painful stimuli during sleep affected the quality of sleep (assessed as sleep latency and fragmentation).

Rationale

Previous studies have found alpha frequency band activity, derived by EEG measurements, to be negatively correlated with cerebral activity in awake subjects (Cook, O'Hara, Uijtdehaage, Mandelkern, & Leuchter, 1998; Marosi et al., 2001). This means that a right dominant cortical asymmetry corresponds to a left dominant alpha power asymmetry. Frontal alpha asymmetry has been consistently related to emotional valence and to basic motivational systems (Davidson, Ekman, Saron, Senulis, & Friesen, 1990;

Wheeler, Davidson, & Tomarken, 1993). A distress-related response has been reported in awake healthy subjects in the form of frontal alpha band changes (left > right) (Harmon-Jones, Vaughn- Scott, Mohr, Sigelman, & Harmon-Jones, 2004; Marosi et al., 2001). These changes have thus been suggested as an objective measure of distress (e.g. expressing negative emotions like frustration, misery, grief, agony or despair). Consequently, frontal alpha frequency asymmetry measured before and after the administration of aversive conditioned and painful stimuli during sleep may be useful as an objective marker of emotional distress in a non-communicative state. Sleep is characterized by sensory detachment from the environment and inhibition of behavioral responses (Carskadon & Dement, 1994). S2 and REM sleep represent a more aroused state than deep sleep (stages 3 (S3) and stage 4 (S4)). At the same time a subject is not as easily awakened as in stage 1 (S1). It is in this study suggested that the relative sensory unresponsiveness and behavioral inhibition of the sleep state can to some extent be compared to pathological or pharmacologically induced silent states or non-communicative states, (Boly et al., 2007). It is on this basis that sleep, explicitly S2 and REM sleep, was used as a model for the experimental hypothesis.

Emotional expression and objective measurement

The science of emotion

Emotions may be defined as “Any short term evaluative, affective, intentional, psychological state, including happiness, sadness, disgust and other inner feelings” (Colman, 2003). The term encompasses a variety of observable behaviors, expressed feelings, and marked changes in both the central nervous system and throughout the body.

Thus, when referring to emotions, one may speak of a subjectively expressed feeling, an emotionally loaded behavior, as well as distinctive somatic responses. One may also refer to more basic motivational programs or systems like the need to seek pleasure, or avoid pain (Rosenzweig, Breedlove, & Watson, 2005). These rather different perspectives of emotion and our rich language for emotional states have made it hard to find emotion variables available to study in a laboratory. Important to the research on emotions has accordingly been the endeavor to structure and standardize the concept (Gazzaniga, Ivry, & Mangun, 2002). Some have categorized emotions viewing them as reactions that vary along a continuum. This calls for meaningful dimensions, one of which is suggested to be the motivational dimension expressed as withdrawal/approach response (Davidson et al., 1990). Investigators have also attempted to define a universal set of discrete emotions that exists across cultures. One attempt to categorize universal and basic emotions explored facial expressions (Ekman & Friesen, 1971; Rosenzweig et al., 2005). With this approach anger, sadness, fear, disgust, surprise, contempt, and embarrassment have been suggested as distinctively expressed emotions (Ekman & Friesen, 1971; Keltner & Ekman, 2000). The debate as to what the labels and numbers of basic emotions should be still stands (Rosenzweig et al., 2005). Despite questions regarding their accuracy, such conceptualizations have been welcomed by scientists as a premise for studying elements of human emotions (Gazzaniga et al., 2002).

Since Darwin's work on the evolution of human behavior it has been argued that emotions may be understood as adaptive modules of motivation and behavior developed through natural selection (Öhman & Mineka, 2001). One very adaptive emotion is the elicitation of fear in regards to danger. Danger calls for rapid changes of perception,

attention, cognition, and behavior (Rosenzweig et al., 2005). The organism needs to be prepared to either fight or flight. An evolutionarily evolved fear module has been suggested as prior to higher cognition, thus enabling an extensive and quick reaction to perceived danger (Tooby & Cosmides, 1990).

When emotional reactions are studied in relation to goal directed behavior (e.g. avoid danger), the close link between motivation and emotion becomes apparent. Many goal directed behaviors happen due to the salient nature of the objective. The consequence of the behavior is linked to a positive emotional state or a reduction in negative valence emotions (Bloom, Nelson , & Lazerson, 2001).

Stress, Distress and negative emotion

Fear causes changes throughout the body that facilitates adaptive behaviors when in danger. The study of these reactions is intertwined with the study of stress. Stress may be defined as a psychological or physiological strain or tension generated by physical, emotional, social, economic or occupational circumstances, experiences or events that are difficult to manage or endure (Colman, 2003). A general definition is unfortunately difficult to render into distinct variables needed for empirical experiments. As with emotions, efforts have been made to clarify terminology (McEwen, 1998; Ursin & Eriksen, 2004).

Cannon recognized early in the 20th century the importance of psychosocial factors in stress reactions (Cannon, 1929). Danger does not have to be an immediate threat (Kirschbaum et al., 1995; McEwen, 1998), as learning, conditioning and the anticipation of adverse stimuli may in itself evoke stress or fear responses (like activation of the autonomic nervous system) (Goldstein & Kopin, 2007). The word distress refers to emotional and

subjective reactions like feelings of hopelessness, suffering, agony and misery (Colman, 2003). It may be seen as a subtype and consequence of stress, elaborating the stress phenomena. While stress may be understood as a global reaction including several biological systems, distress is a psychological and emotional reaction that is not necessarily contingent to the somatic dimensions (Goldstein & Kopin, 2007).

Cortisol and galvanic skin response (GSR) are important measurements of the general stress and fear reaction. However, objective measurements of subtle emotional expressions are difficult to obtain given that these are better conveyed by subjective verbal expression (Kappesser, Williams, & Prkachin, 2006). In some circumstances facial expression has been used to provide an objective framework to observe emotions (Kappesser & Williams, 2002) and different clinical manuals have been produced (FACS, EMFACS scales, CHEOPS and OPS, CFCS) (Gilbert, 1999; Magai, 2002, 2006; Suraseranivongse, 2001). However, these methods often underestimate feelings of distress (Kappesser & Williams, 2002; Kappesser et al., 2006).

Emotion and conditioning

Survival has depended on systems that manage dangers (Öhman & Mineka, 2001). With more sophisticated nervous systems, survival is achieved through more refined and selective mechanisms such as inborn defense responses, Pavlovian conditioning, instrumental learning, and eventually cognition and deliberation (Öhman & Mineka, 2001). In Pavlovian conditioning, also known as classical conditioning, existing responses in the organism are used to achieve learning. An unconditioned stimulus (UCS) that evokes a definite response (e.g. electric shock elicits stress and fear) is paired temporally with an

otherwise neutral stimulus (e.g. a sound). The neutral stimulus becomes a cue for the UCS. By using the contingency between such cues and for instance potential pain/danger, the fear response can be conditioned to the cue, now called a conditioned stimuli (CS) (Schwartz, Wasserman, & Robins, 2002). If a neutral stimulus is paired with an UCS it is referred to as a CS+. If an otherwise neutral stimulus is presented without an UCS, it is defined as a CS-. Fear is recognized as potent in conditioning, as a conditioned response may be easy to achieve but difficult to get rid of, even if the fear is irrational (Schwartz et al., 2002).

In the present project, conditioning was accomplished in two distinct ways in two different experimental groups. One procedure entailed a classical fear conditioning protocol wherein electric shock constituted the UCS. Also, images of strong negative emotional valence were used as UCS. Both procedures were chosen to ensure conditioning of negative valence emotion. Additionally, a third experimental group did not receive conditioning but mild electric shocks during sleep. Both conditioned and pain stimuli were expected to elicit emotional distress. The GSR represents changes in electrical conductive properties of the skin. Changes are a function of the autonomic and basal ganglia- limbic control circle's (including amygdala) modulation of sweat gland activity (Hugdahl, 2001). Hence, emotions and distress significantly affect the electrodermal response. For this reason GSR is extensively used to show establishment of a conditioned response and stress (Öhman, 1993), and was accordingly used in this study.

While cortisol and GSR, are closely linked to the measurement of stress reactions (fight or flight), EEG have been extensively used in studies of emotional states and behavioral modules (like approach and withdrawal) (Allen, 2004). The present study looks

at event-related responses acquired from EEG measurements and results will be interpreted in relation to emotion and motivational direction.

Frontal alpha asymmetry as a reflection of emotional valence

In 1978 Davidson presented findings suggesting that negative valence emotions were linked to asymmetric frontal activity (Davidson, Schwartz, Saron, Bennet, & Goleman, 1979). Emerging evidence in the eighties proposed that perception and expression of emotions were represented asymmetrically in the cerebral hemispheres. Three different hypotheses emerged. Emotions were possibly better recognized and efficiently processed by the right hemisphere. Secondly it was suggested that control of emotional expression and related behaviors mainly took place in the right hemisphere. A third suggestion was that the right hemisphere was specialized for dealing with negative emotions, while the left mainly processed positive ones (Silberman & Weingartner, 1986). Extensive evidence suggested that there was a right hemispheric dominance for processing of negative valence emotions (Silberman & Weingartner, 1986). Research has also considered that lateralisation possibly constituted a stable individual variable (Davidson & Fox, 1989; Jacobs & Snyder, 1996).

The attention of this field has been directed to activity in the frontal lobes (Marosi et al., 2001). This may be related to the proposition that left and right prefrontal regions are related to a withdrawal/ approach (W/A) system discussed later in this text (Davidson et al., 1990). It has furthermore been a proposed and to some extent recognized that EEG frequencies in the alpha band (8-13 Hz) are inversely related to the level of cortical activity in awake subjects (Cook et al., 1998; Marosi et al., 2001). Increased alpha activity in

wakefulness may be observed when underlying systems disengage from active processing, thus a decrease in alpha power signifies an increase in cortical activity (thus left > right hemispheric activity is expressed as Right >Left alpha activity) (Coan & Allen, 2004). Studies have, conversely, also looked at other frequency bands (Aftanas, Reva, Savotina, & Makhnev, 2006; Marosi et al., 2001; Rusalova & Kostyunina, 2004).

The differential frontal hemispheric brain activity often referred to as frontal asymmetry has become a common way to measure emotional responses (Harmon-Jones et al., 2004) and individual predispositions (Jacobs & Snyder, 1996). Prolonged asymmetric frontal alpha activity or activation can in this respect be seen as individual variances in temperament, emotional style and risk of developing emotional disorders, whereas a temporally limited asymmetry can be interpreted as an emotional response (Coan & Allen, 2004). Negative affect has been related to greater right than left cortical activity both in baseline (Davidson & Fox, 1989) and stimuli evoked conditions (Tomarken, Davidson, Wheeler, & Kinney, 1992b; Wacker, Heldmann, & Stemmler, 2003). Greater right than left alpha activity (accordingly greater left cortical activity) has been suggested to express positive affect (Tomarken, Davidson, Wheeler, & Doss, 1992a; Wheeler et al., 1993). However, in sleep, alpha activity may be related to increase in activity. Nevertheless, asymmetric hemispheric representations of several power bands also during sleep, has been shown in depressed patients (Armitage, 1995). It has been suggested that as in waking EEG, an increase of asymmetry may be observed both as reactions to external stimulation, as well as trait like tendencies in sleep (Armitage, 1995).

More recently, there has been an increased focus on motivational direction rather than emotional valence in the research on hemispheric asymmetry. Asymmetry in frontal

brain activity may not only reflect the emotional valence, but the operative motor response either to withdraw (left > right alpha power) or approach (right > left) (e.g. fear triggers withdrawal whereas anger or love triggers approach) (Coan & Allen, 2003). Not all emotions associated with approach are of positive valence. Increased left frontal asymmetry (e.g. right frontal alpha asymmetry) is found in relation to anger responses (Harmon-Jones et al., 2004).

It has been pointed out that there are conceptual similarities between the W/A model and Gray's behavioural activation (BAS) and behavioural inhibition system (BIS). Some findings suggest that this model of behavioural activation better explain hemispheric asymmetry (Wacker et al., 2003). The mechanism responsible for the generation of alpha asymmetry remains speculative.

Nevertheless, a shift of emotion is generally thought to elicit differential hemispheric lateralization. If all aversive stimuli generate an indistinct feeling of distress during sleep, responses similar to the ones observed in wakefulness may be obtained. It is believed that under no circumstances may the presentation of aversive conditioned and painful stimuli elicit positive valence emotions either in waking or in sleep. The most likely brain activity consistent with frustration, grief or fear, could be generated under such circumstances and expressed as left > right alpha asymmetry. However, we cannot exclude that some subjects may respond with anger, possibly a right > left alpha asymmetry. Also, emotionally loaded images of negative valence may have a different impact on subjects than pain stimuli, which may possibly generate more substantial responses compared to the pain-conditioned stimuli. Nonetheless, it is expected that if similar generated event-related changes in emotional state is achieved, it will be similarly reflected in change of frontal

alpha power. This is highly dependent on proper conditioning and administration of stimuli. A general response regardless of stimuli, may additionally suggest that alpha asymmetry makes up a robust measurement of distress, perhaps also of practical use outside the experimental restrictions of this study. This might be conditions where occurrences of stressful emotions can be anticipated (e.g. intensive care units, post- surgery patients still under anaesthesia and more)

Sleep

Sleep is generally defined as a reversible behavioral state of perceptual disengagement from, and unresponsiveness to the environment. Additionally, sleep is typically, accompanied by postural recumbence, behavioral calmness and closed eyes (Carskadon & Dement, 1994). Human sleep can be classified into two states; REM sleep, and Non REM (NREM) sleep. The latter is divided into S1, S2, S3 and S4. S3 and S4 are commonly referred to as deep sleep. The states are defined using polysomnographic (PSG) recording which among others comprises EEG recordings (see PSG procedures, method section).

The EEG of a waking human is characterized by desynchronized, mixed frequency activity of low amplitude/ voltage, whilst quiet wakefulness with eyes closed is typically characterized by alpha activity (see methods for band definition). EEG during sleep is characterized by lower frequency and higher amplitude. However sleep EEG also comprises periods of activity similar to that of quiet wakefulness but with other elements not corresponding to a fully awakened state (Rechtschaffen & Kales, 1968). Initially it was believed that the high cognitive content of dreaming was an exclusive marker of REM

sleep. However, it was found that mentation also takes place during non-REM sleep (Cavallero, Cicogna, Natale, Occhionero, & Zito, 1992; Pivik, 1994; Portas et al., 2000). Sleep is thus a state of mental activity, but it is behaviorally, and functionally different from wakefulness. Mental activity is furthermore varied in different stages of sleep. S2 and REM sleep especially, may include periods of higher frequency activity, alpha frequencies included, hence representing a more active state than S3 and S4 (Carskadon & Dement, 1994).

The transition from wakefulness to sleep is often difficult to pin point. Simple behavioral responses (e.g. tapping two fingers when stimuli are presented) have been shown to persist for a short time into S1. In regards to auditory stimuli, reaction time becomes longer when subjects are very relaxed awake, and it disappears at the transition into sleep. However, individuals' sensory input is not entirely blocked during sleep. Sensory processing does at some level continue after sleep onset, albeit with a selective responsiveness to meaningful stimuli (Perrin, García-Larrea, Mauguière, & Bastuji, 1999; Portas et al., 2000). There is furthermore evidence in rodents that conditioning takes place during sleep (Coenen & Drinkenburg, 2002). Persistence of sensory processing has also been observed in comatose patients (Boly et al., 2007; Laureys et al., 2000; Siva, 2006) and patients under anesthesia (Magarey & McCutcheon, 2005; Orser et al., 2007; Sandin et al., 2000). Hence, it was in this study suggested that the relative sensory unresponsiveness and behavioral inhibition of the sleep state (involving lack of communication to the outside world), can to some extent be compared to pathological or pharmacologically induced non-communicative states. It was on this basis that sleep was used as a model for the experimental hypothesis.

Hypotheses

In this study, three different groups of subjects were exposed to neutral stimuli as well as to one of three types of aversive stimuli during sleep (see method section). Two different types of conditioned stimuli, as well as mild electric shocks were used to induce feelings of distress. The main hypothesis of this study depended on a proper conditioning and administration of stimuli.

Frontal alpha asymmetry was used as a measurement of the responses to aversive stimuli. While alpha activity has been associated with reduced activity in the wakeful brain, alpha intrusions in sleep is a sign of heightened activity, compared to the rhythmic slow wave sleep of S3 and S4. However, it was change in frontal alpha asymmetry not general changes in the quantity of alpha activity that was the main interest of this study.

In the present study it was expected to find a significant change in frontal alpha asymmetry to aversive compared to neutral stimuli during sleep, regardless of state (S2, REM sleep and short awakenings, if any). Furthermore, frontal alpha was expected to change to a more dominant left alpha activity (hence more right frontal cortical activity) consistent with the dominant response to the majority of negative emotions (Aftanas et al., 2006; Jacobs & Snyder, 1996; Marosi et al., 2001).

Sleep latency and the number of arousals and awakenings (e.g. sleep fragmentation) during the night of stimuli presentation, are hypothesized to be moderately affected in relation to stress reactions since the aversive stimulation was administered only for brief periods during sleep and therefore it may not be sufficient to produce a long-lasting effect evident in the sleep data.

Method

Ethical Considerations

The National Committee for Medical Research Ethics in Western Norway approved the study prior to its implementation. The study was also reported to the Norwegian Social Science Data Services. All subjects signed an informed consent before participating in the study (see appendix VI).

Subjects

In total 34 volunteers between twenty and forty years of age were recruited. All subjects were screened for medical and sleep disorders (see screening instruments and experimental measurements and questionnaires section and appendix I). They were further given written instructions to keep a sensible sleep routine during the week before the experiment (e.g. to avoid going to bed after midnight or wake up before 5 in the morning) and to refrain from caffeine at least six hours before each experimental session. Likewise the consumption of alcohol was restricted twelve hours, and nicotine and foods one hour before each experimental session (see appendix VI a).

The subjects spent three nights at the sleep laboratory. All subjects were woken up at 06:45 each morning. The first night ensured that subjects habituated to the laboratory conditions(Agnew, Webb, & Williams, 1966). Night two functioned as a baseline night for sleep and stress data, both subjective (diaries and analogue scales) and objective (PSG and cortisol saliva samples). During night three, the experimental recording night, subjective and objective measurements were obtained (see screening instruments, experimental

measurements and questionnaires). During sleep the third night, different stimuli were administered depending on what group the subjects were assigned to.

Groups and experimental conditions

Subjects were randomly divided in three groups. As shown in figure 1, all groups received one of the aversive stimuli both during S2 and REM sleep on the third experimental night. Group 1 received aversive emotional conditioning with images of strong negative valence content as the UCS. Group 2 received pain conditioning with mild electric shock as the UCS. Group 3 received pain in the form of mild electric shock during sleep.

Earlier in the evening of the second recording night, an individual pain threshold was assessed for each of the subjects in group 2 and 3 (see Evaluation of pain threshold section). Before the start of the third recording night Group 1 and 2 subjects underwent a Pavlovian model of conditioning (see Conditioning and administration of stimuli section). On the third night these subjects were exposed to the CS+ and to CS- (sounds of different pitch) (see conditioning and administration of stimuli). During the third recording night, group 3 was in addition to mild electroshocks also exposed to a neutral control sound (sounds of different pitch) (see conditioning and administration of stimuli). The aversive stimuli and the control stimuli were administered interchangeably in all three groups.

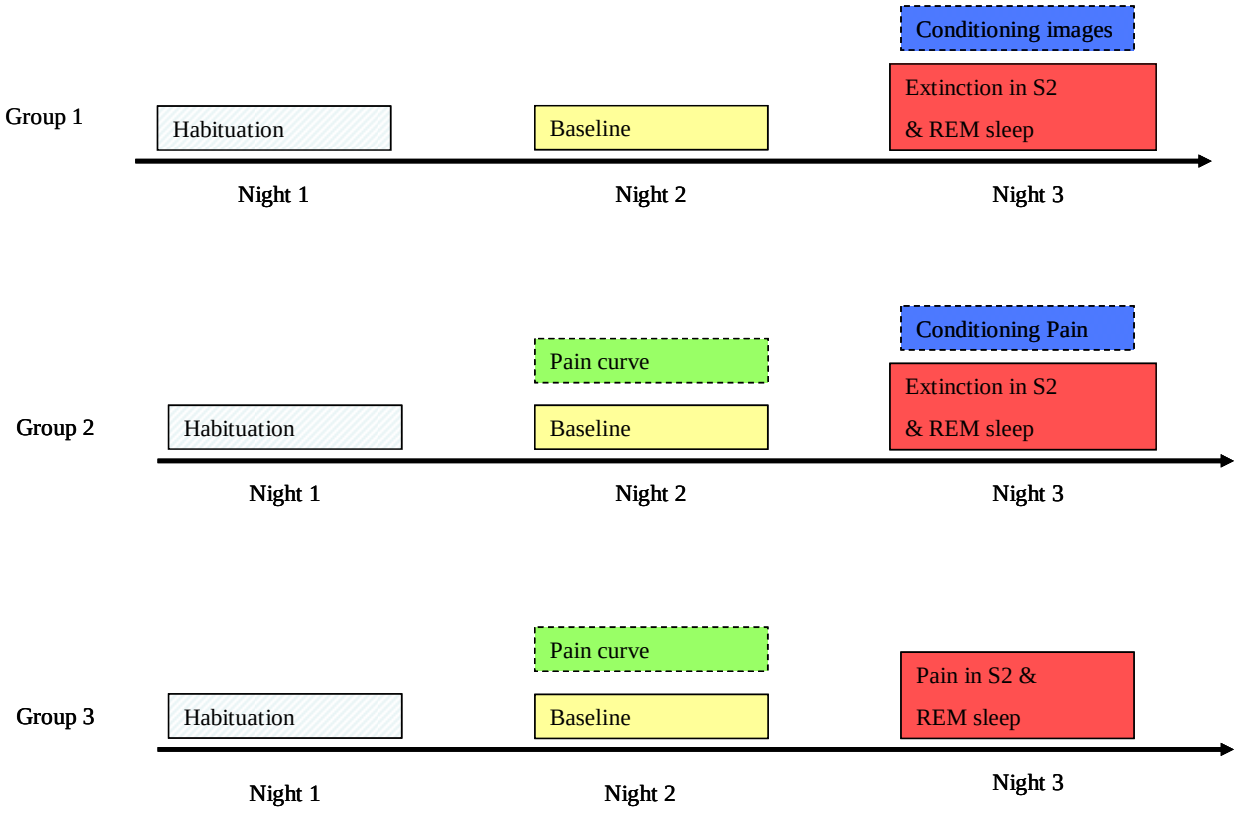

Figure 1

Experimental conditions for the three groups

Conditioning and administration of stimuli

The conditioning protocol used in this study was an adaptation from Carter and collaborators. CS+ presentations were paired with an unconditioned stimuli being either aversive images or electrical shocks. The CS- sound was presented without aversive stimuli (Carter, Hofstötter, Tsuchiya, & Koch, 2003).

The conditioning that took place the third night consisted of three phases: habituation, acquisition and extinction. In the habituation phase, subjects received 2 presentations of the CS+ and 2 of the CS- to familiarize with the stimuli. During the acquisition phase, subjects received 4 presentations of CS+ and 4 of CS-, randomly

presented, in total 8 stimuli. Successful establishment of acquisition was assessed using GSR (see GSR section). The extinction phase took place during S2 and REM sleep. Subjects received 8 presentations of CS+ and 8 of CS- randomly, in total 16 stimuli. The software E prime was used both for administration of pain stimuli and conditioning of subjects (Sætrevik, 2007).

Evaluation of pain threshold

An individual pain curve was assessed before the second recording night for subjects in group 2 and 3 (subjects receiving pain conditioning or pain during sleep). The subjects were asked to self-rate the experience of mild electric shocks administered to the left forearm using an analogue scale ranging from zero to ten. Zero was equivalent to no sensation of stimuli at all, whereas ten was the worst pain imagined. To prevent sensitization, shocks were administered in a nonlinear increase of potency, starting with a very barely perceivable stimulus (1mV, 10 pulses). In total 10 shocks were delivered. Stimuli self-rated between four and five was set as the subject's pain threshold, and used both in pain conditioning (group 2 subjects) and pain administration during sleep (group 3 subjects).

Sounds

The CS+ and CS-/neutral stimuli were digitally produced sounds of different pitch. One was 1000 Hz, the other was 500 Hz, both ranging between 55 and 65 decibel lasted two seconds. Whichever sound chosen to constitute CS+ or CS- was randomized between

subjects. The sound that would constitute the neutral control stimuli administered to group 3 was also randomized.

Images

The images used in the negative emotions conditioning (group 1) were taken from the International Affective Picture System (Lang, 1997). The four images selected for the conditioning protocol were rated as most aversive and emotionally upsetting by a group of six independent judges not otherwise partaking in the experiment.

Administration of painful and conditioned stimuli during sleep

Administration of both painful and conditioned stimuli was carried out when S2 and REM sleep were fully established. The first administration of stimuli was carried out in S2 after a period of S3/S4 sleep had occurred. The second administration occurred in established REM sleep. Stimuli were not administered unless 3 minutes of stable S2 or REM sleep had been observed. The intensity of the painful stimuli administered during sleep in group 3 was equal to the subjects' individual waking pain threshold and lasted 0.5 seconds. Since an individual's pain threshold is higher during sleep, it was assumed that such stimuli should be strong enough to elicit a response, but not to the degree of completely awaken the sleeper (but for the occasional occurrence of microarousals). To find the subjects' individual pain threshold and to apply it during sleep proved more difficult than assumed (see discussion and appendix II).

Screening instruments, experimental measurements and questionnaires

Before partaking in the experiment, subjects were screened based on their general health, (general health questionnaire) and sleep habits (Pittsburgh Sleep Quality Index, and Epworth sleepiness scale)(Buysse, Reynolds, Timothy, Berman, & Kupfer, 1989; Johns, 1991) (see appendix I). The subjects were also required to complete a sleep diary during the week before participation, ensuring reasonable sleeping behavior prior to the experiment (e.g. to avoid going to bed after midnight or wake up before 5 in the morning). Sleep deprivation or equally poor sleep practice entailed exclusion of that participant. All questionnaires administered were Norwegian editions (Beiske, Kjelsberg, Ruud, & Stavem, 2009; Pallesen et al., 2005). After completing the experiments, all subjects were screened for periodic limb movement disorder (PLMD), and sleep apnea (see Polysomnographic and Physiologic Recordings).

Scales and questionnaires were also administered to participants both before and during the experiment. NEO PI–R, the standard measurement of the Big Five traits was administered (Costa, 1992). The subjects were furthermore asked to provide a spontaneous report of remembered dreams on tape, as well as to fill in a standard dream questionnaire (Schredl, 2002) and dream diary. The subjects also reported the experience of their sleep, answering a standardized questionnaire. An additional question regarding experienced sleep quality was included (for all questionnaires, see appendix I). The subjective and objective experience of stress was assessed before every recording night and the following morning, expressed in an analogue scale ranging from zero (no stress at all), to ten (extreme stress) and cortisol saliva samples. These results are not reported in this text, but will be available in a separate publication.

Galvanic Skin Response

In this study GSR was used to assess the acquisition of conditioning in group 1 and 2 subjects. In addition, GSR was measured throughout the night to further monitor event-related changes (extinction phase). Electrodes were attached to the second phalanx of the first and second fingers of the dominant hand. Signals were acquired using a ML116 GSR Amplifier from AD Instruments interfaced with Power Lab Software. Responses were significant if they occurred between 1 to 5 seconds after the stimulus, with a change in amplitude of at least 0.05 mV (Hugdahl, 2001). Also, the fluctuation in amplitude must have decreased $1/4^{th}$ to consider the occurrence of another response to a stimulus (Nordby, personal communication, April, 2009). Thus in this study, a good acquisition entailed a significant response to aversive stimuli and in contrast a non-significant response to the neutral control stimuli, and similarly a good extinction phase entailed a significant response to the CS+.

Polysomnographic procedures

The standardized 10/20 system was used for EEG electrode placement (Jasper, 1958). This system covers the placement of electrodes throughout the scalp. The locations are determined by reference points nasion and inion. From these points, the skull perimeters are measured in transverse and median planes, divided into 10% and 20% intervals. Each position is named according to region placement (e.g. Frontal polar = Fp; Frontal = F; Central = C; Parietal = P; Auricular = A and Occipital =O). Even and odd numbers specify hemisphere and antero- posteriority (e.g. right = even; left = odd) (See illustrations,

appendix V). Electrodes were manually placed and held in place using tape and a head net. All electrode impedances were less than 5000 ohms. Embla hardware (Embla N7000, Polarmed, Norway) and Somnologica software (Somnologica studio 5.5) was used for sleep recording. Signals were recorded at a sampling rate of 256 Hz.

Standard recommendations for PSG recording requires a minimum of one EEG lead, C3 or C4, referenced to a contra lateral auricular electrode (A1/A2), consequently C4/A1 or C3/A2 (Rechtshaffen & Kales, 1968). However, O1/A2 or O2/A1 is usually recorded as well (Carskadon & Rechtschaffen, 1994). Additionally, eye movements measured by two electro oculogram (EOG) leads and submentalis muscle movements measured with electromyogram (EMG) leads are required.

However, the scope of this project required a more comprehensive PSG recording system, suitable for assessment of cortical activity in multiple EEG leads. In addition to standard recommendations, this study recorded EEG activity on following leads: Fp1/2, F3/4, F7/8, A1/2, T3/4, C3/4, P3/4 and O1/2.

Additional electrodes for screening PLMD and breathing disorders required the use of airflow (nasal pressure capsula, thermistor), arterial oxygen saturation, respiratory effort (abdominal and thoracic bands), electrocardiogram (ECG) channels, pulse rate and tibialis surface EMG leads (Redline et al., 2007; Walters et al., 2007).

Data Analyses

Sleep staging

Sleep was scored in standard epochs of thirty seconds. Each epoch was scored based on the dominant sleep stage in that epoch (more than fifty percent, e.g. 15 seconds (Rechtschaffen & Kales, 1968)

Active wakefulness is characterized by high frequency and low amplitude rhythms (beta, gamma frequency) on the EEG leads accompanied by high muscle tone and high frequency eye movements. Quiet wakefulness is dominated by alpha activity (8-13 Hz). S1 is recognized by a decrease in alpha and predominantly theta activity (4-7 Hz). S2 also contains theta waves, and additionally K-complexes (large amplitude waves, 0,5–2 Hz > 75 μV)) and sleep spindles (diamond shaped clusters of higher frequency activity 11-16Hz, usually 12–14 Hz). S3 and S4 are both characterized by slow wave delta, 0,5–2 Hz > 75 μV, 20 and 50% respectively). Non-REM sleep is furthermore recognized by low EMG activity and a minimum of eye movements. Arousals were defined as an abrupt shift in EEG frequency, lasting at least 3 seconds, with at least 10 preceding seconds of sleep. REM sleep arousals were scored only if accompanied by increased EMG submentalis activity.

Screening of sleep pathologies

Subjects were screened for sleep apnea and PLMD to avoid that data analysis might include pathologic data. Obstructive apnoeas were scored when airflow dropped below 10% of the reference amplitude for more than 10 seconds. Hypopnoeas were scored when airflow dropped below 30% for more than 10 seconds with subsequent oxygen desaturation of 4% (Thorpy, 1990). Leg movements was scored when lasting between 0.5 sec and 10

seconds, and showed increased amplitude of more than 25 % from resting EMG. The leg movements were scored as periodic if they formed a series of at least 4 movements between 5 and 90 seconds apart (Thorpy, 1990). Exclusion criteria were defined by an apnea-hypopnea index greater than 5 or a PLM index greater than 15.

Signal processing of power spectrum raw data

To attain EEG signal in metrics, raw signals were transformed from a temporal to a frequency based representation in form of a power spectrum (Allen, Coan, & Nazarian, 2004). For this study, power spectrum data were acquired for alpha bands (8- 13 Hz) in EEG leads F3 and F4. As shown in figure 2, two second epochs of before and after stimuli administration were considered for data analysis. Data acquired during stimuli presentation were not utilized due to difference in stimuli duration. A fast Fourier transform, Welch method, overlapping data windows by 50 %, was used calculating power spectrum data.

Alpha power values were natural log transformed (Ln) to normalize the data distribution, avoiding a skewed data set. An index of hemispheric lateralization was obtained by subtracting LnF3 from LnF4 values (index = Ln F4 - Ln F3). This corrects for some of the individual variance in overall alpha power and skull thickness that otherwise could be confounded with the size of the asymmetry (Eshel, Witman, Rosenfeld, & Abboud, 1995). The difference of before and after stimuli administration were also transformed into an index (after aversive stimuli – before aversive stimuli, or after neutral stimuli – before neutral stimuli), enabling a focused frame to study the difference between the neutral and aversive stimuli.

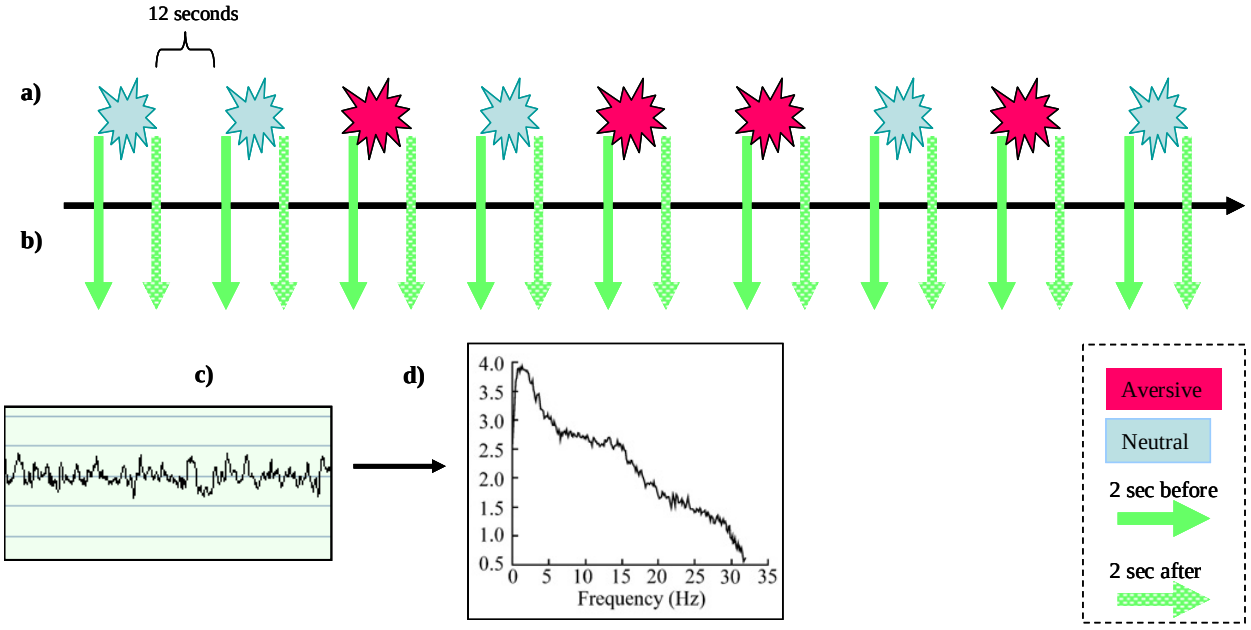

Figure 2

Temporal windows of EEG waves used in subsequent Power spectrum analyses. (a) Data from stimuli presentation was the basis for power spectrum analysis. (b) Power spectrum data used in statistical analyses was alpha power of 2 second epochs before and after stimuli. (c) These data was transformed from EEG waves with a time based x- axis, (d) to a Power spectrum, a frequency based representation of the data.

Statistical analyses

Out of the 34 subjects who participated in the study, only 30 completed the experimental procedure (e.g. three nights sleep recordings, pain threshold assessment, conditioning and successful administration of stimuli in both stages S2 and REM sleep). Three subjects were excluded as a result of either EEG data being two standard deviations from the mean of given group or because of technical problems with time codes. One subject was additionally excluded from sleep data analyses because of a difference from group values of more than 2 standard deviations (see appendix II). Hence, the first set of

alpha index analyses were based on a total of 27 subjects (9 in each group), and sleep analyses were based on 26 subjects (8 in group 2) (see appendix II). Short awakenings were not excluded from the data set as they did not appear to differ from the EEG data of sleeping subjects, nor from the subjects' own frontal alpha asymmetry in sleep. No subjects were excluded due to respiratory sleep disorders or leg movements, or other health related issues (see appendix II b).

Alpha index analyses. Repeated measurements MANOVA were carried out including the following factors: groups (as independent variable), number of stimuli (4 repetitions), CS+/CS- (2 levels) and sleep stages (either 2 levels, looking separately at S2 and REM sleep or 1 level analysing effect of aversive stimuli regardless of sleep stage). There were a few cases where some raw data were removed or were missing due to artifacts in sleep (e.g., body movements or signal error), and error in stimuli administration. The relative data points represent only 11 of a total of 1728 values. These values were replaced using a regression analysis.

Further exclusions of 8 subjects were carried out after finding some inconsistencies in the acquisition phase of the conditioning procedure and in the estimate of pain threshold (see methodological issues, discussion section and appendix II a). A repeated measurement MANOVA was conducted with all remaining subjects for each group separately. Finally, one MANOVA analysis CS+/CS (2 levels) and number of stimuli (4 repetitions) was conducted for all remaining subjects considered as one distress group.

Sleep data analysis. Sleep latency, arousals and awakenings in all 3 groups were compared looking at descriptive mean and standard deviation values, as well as doing repeated measurements MANOVA, with two levels (night 2 and night 3).

GSR data analysis. GSR data analysis was provided by Tone Blågestad and will be available in a separate publication elsewhere

One-tailed probability values were used in cases where there were strong experimental hypotheses (e.g. change in frontal alpha asymmetry). Otherwise, significance was accepted at $p<0.05$, two tailed.

Results

Frontal Alpha asymmetry

In the present study it was anticipated that an increase in frontal alpha asymmetry, measured as a lateralization F4 / F3 index, would occur as a response to aversive stimuli (image/pain conditioning and pain). This hypothesis was first tested looking at the differential response to CS+ or pain compared to CS- or a control stimuli, in S2 and REM sleep.

When looking separately at S2 and REM sleep, the change in frontal alpha asymmetry was close to significant ($F_{1, 27} = 2.86$, p= 0,052 one-tailed). Sleep stages showed no significant difference in frontal alpha asymmetry ($F_{1, 27} = 0.33$, $p = 0.57$). Thus, it was reasoned that both sleep stages could be treated as one homogenous factor i.e. sleep. Importantly, also brief awakenings evoked by the stimuli administration did not change a subject's asymmetry response; hence both sleep stages as well as short awakenings were pooled in the analysis.

When looking at the frontal alpha asymmetry regardless of sleep stage, significant change in frontal alpha asymmetry between control sound and aversive stimuli (CS+ or

Pain) was present($F_{1,27}$= 4.48, p = 0.022 one-tailed). However, as evident in figure 3, the groups had a differential response to the stimuli (control stimuli, CS-, or aversive stimuli, CS+/Pain). A closer look at each group was evidently necessary.

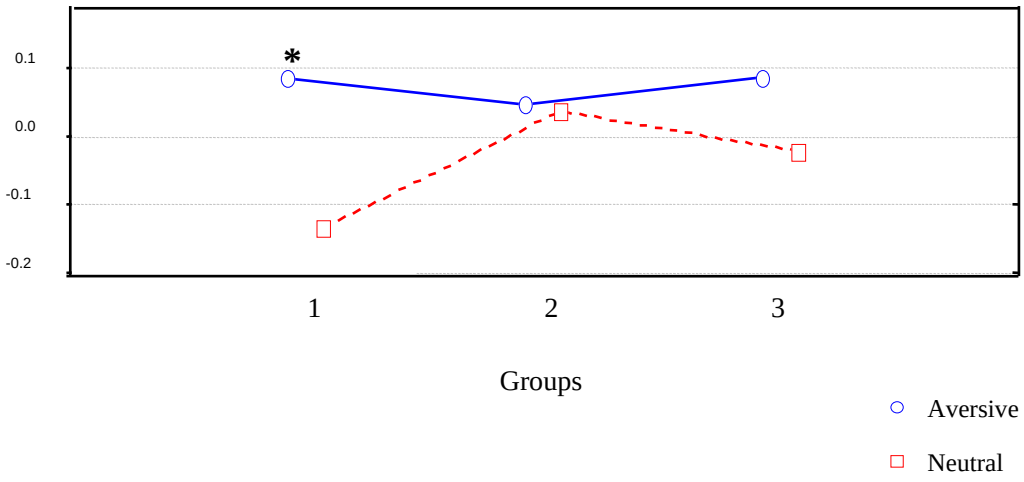

Figure 3

Frontal alpha asymmetry in neutral or aversive conditions regardless of sleep stage. 1 = aversive images conditioning, 2 = pain conditioning, 3= pain during sleep. Results are presented as mean, * p<0.05 compared to neutral stimuli.

The strong response in group 1 seemed mainly responsible for the significant outcome of this analysis, as only group 1 showed a clear and significant change in alpha asymmetry ($F_{1, 9}$ = 7.152, p = 0,013 one-tailed). As evident in figure 4, all subjects in group1 show a consistent change in frontal alpha asymmetry. In one left- handed subject the lateralization of response is reversed (see appendix II c). The two other groups (pain conditioning and pain stimuli) show no significant change in frontal alpha asymmetry.

Thus, further investigation was required into factors that may have contributed to the deficient responses to the aversive stimuli.

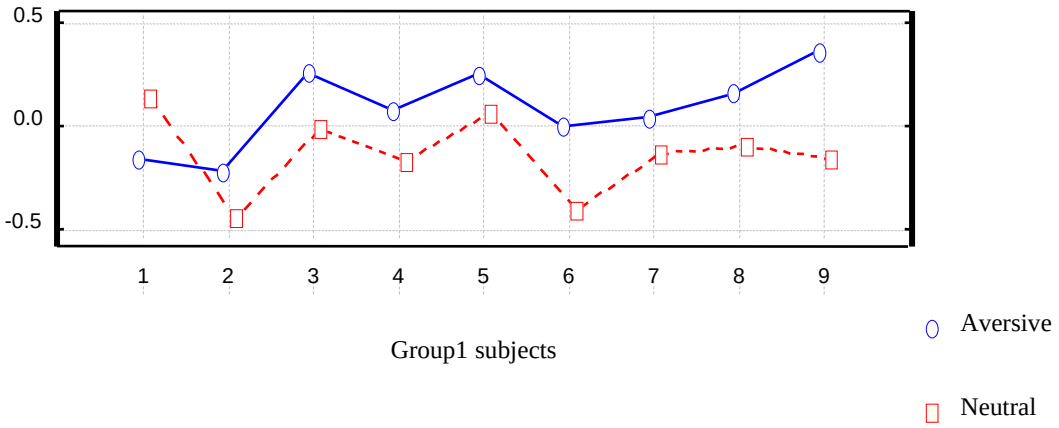

Figure 4

Change in frontal alpha asymmetry in group 1 (aversive image conditioning). Note that subject 1 was left handed.

It soon became evident, when looking at the GSR and pain curve data, that some problems related to pain threshold assessment and subsequent conditioning and pain administration, had affected the results for some subjects in group 2 and 3 (for further details see methodological issues, discussion section and Appendix II a and b). When excluding the subjects who were not conditioned or had not received adequate pain administration, a significant change in the frontal alpha symmetry (left > right) was found, despite the low number of subjects ($F_{1, 5} = 6.16$, $p = 0.028$ one-tailed, and $F_{1, 4} = 15.45$, $p = 0.009$, one-tailed respectively) (see figure 5 and 6). The inversed response in subject 2 in group 2 is not due to left handedness, as observed in group 1 (see appendix II c).

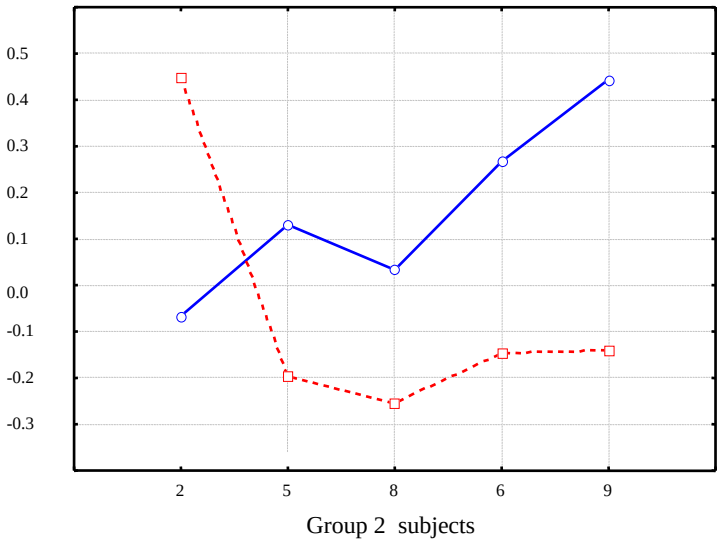

Figure 5

Change in frontal alpha asymmetry in group 2 (pain conditioning), after exclusion of 4 subjects not conditioned.

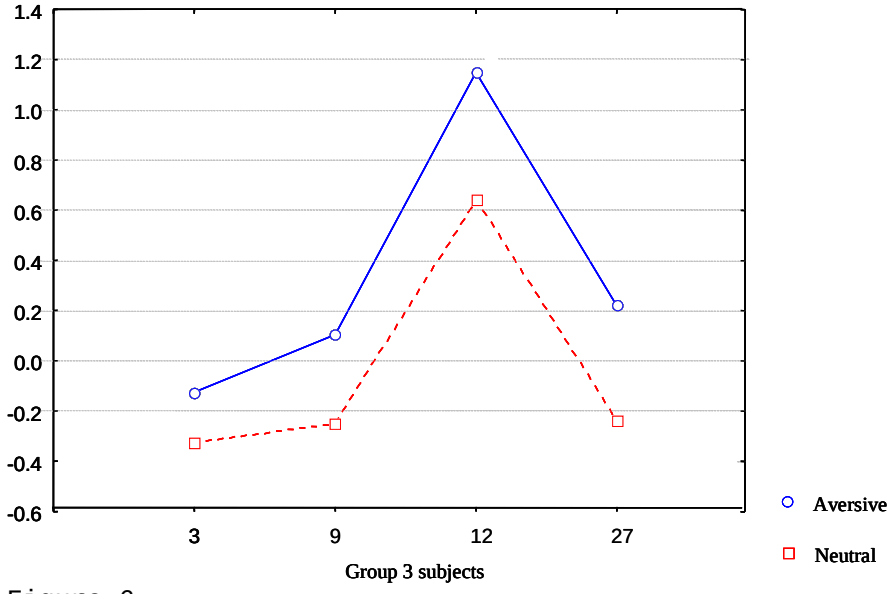

Figure 6

Change in frontal alpha asymmetry in group 3 (pain during sleep) after exclusion of 5 subjects not administered pain stimuli.

After these exclusions, all remaining subjects (e.g. subjects in all groups who were exposed to aversive stimuli of different nature) were treated as one distress group (N= 17).

As shown in figure 7, the change in frontal alpha asymmetry between control stimuli and aversive stimuli is further strengthened in significance ($F_{1, 17} = 32.22$, $p = 0.00003$ one-tailed). All subjects with reversed change in asymmetry (right > left) were left handed.

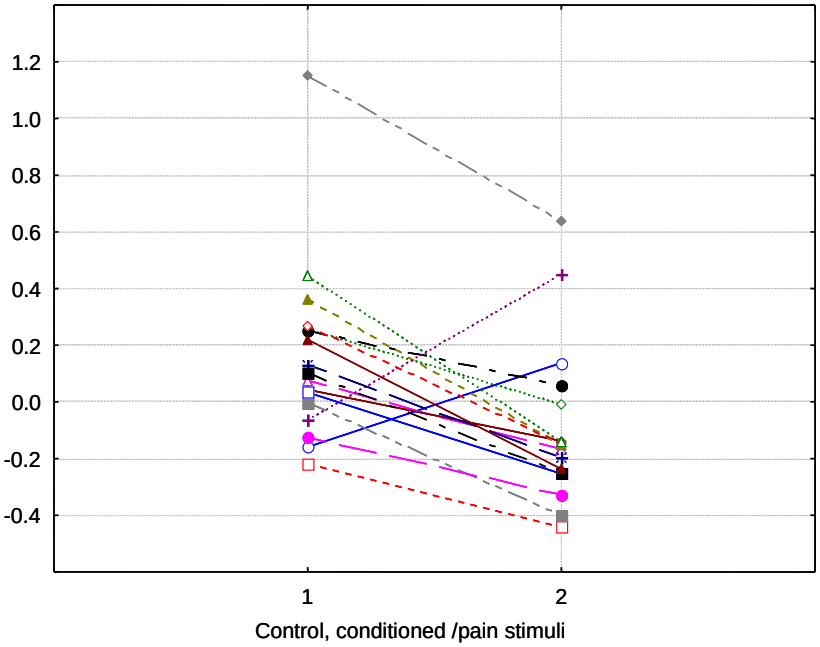

Figure 7

All subjects from the three groups who underwent appropriate aversive stimulation. Note that one of the two subjects having inversed asymmetry values was left-handed.

Sleep results

It was considered if aversive stimulation would affect sleep latency and fragmentation as a consequence of stress. Analyses were carried out according to the exclusions previously conducted. There are no significant differences in sleep latencies between groups or between recording night two and night three ($F_{2, 16} = 1.79$, $p= 0.2$, and $F_{2, 16} = 2.41$, $p = 0.13$). Sleep latency remained consistent in each subject from one night to the other (see appendix III b). Moreover, as shown in Table 1, results may have been

confounded by high variance in group 2. It was also considered that aversive stimulation may increase sleep fragmentation. Fragmentation of sleep can be expressed both as number of awakenings as well as total number of arousals. There are no significant group or recording nights differences in either awakenings ($F_{2, 16}$, 0.51 p = 0.49 and $F_{2,16} = 1.74$, p = 0.21), or arousals ($F_{2,16} = 0.33$, p= 0.58 and $F_{2,16} = 042$, p= 0.67,).

Table 1

Descriptive data; sleep latencies night 2 and 3 for each group

	Groups	Mean	Std. Deviation	N
Sleep latency Night 2	Group 1	10.67	4.92	9
	Group 2	18.88	12.49	4
	Group 3	9.00	4.24	4
	Total	12.21	7.73	17
Sleep latency Night 3	Group 1	11.89	7.54	9
	Group 2	17.00	9.06	4
	Group 3	5.63	3.77	4
	Total	11.62	7.92	17

Galvanic skin response

GSR measurements were obtained both during acquisition and extinction phases in groups 1 and 2, as well as during pain administration during sleep in group 3. GSR was reliable in determining if a successful acquisition had taken place. There was no consistent GSR response to the conditioned stimuli during sleep (Extinction phase). GSR results were provided by Tone Blågestad and will be available in a separate publication elsewhere.

Discussion

Main findings

This study aimed to find a measurable physiological response linked to the human experience of distress in conditions where no overt communication is possible. The effect of the administration of different aversive stimuli during sleep was assessed as changes in frontal alpha asymmetry compared to responses to neutral control stimuli. In addition, it was considered that aversive stimulation may also affect sleep latency and fragmentation as a possible consequence of stress.

Results show that frontal alpha asymmetry changed consistently (left > right) as a function of aversive image conditioning in group 1 (aversive images conditioning). At first examination of the other two experimental groups (pain conditioning and pain during night) did not show such effects. However, at a closer look it was found that several subjects in groups 2 and 3 were neither conditioned nor received appropriate levels of pain stimulation during sleep. When the subjects that had not been properly exposed to experimental conditions were excluded from data analysis, aversive stimulation resulted in significant changes in frontal alpha asymmetry (left > right) also in groups 2 and 3. A reversed change in asymmetry was observed in left-handed subjects (right > left).

Neither different sleep stages nor brief awakenings, evoked by the stimuli, seemed to change a subject's asymmetry response; hence these data were pooled in the analysis. It appears that the index shows changes in frontal alpha asymmetry regardless of the state of the brain before the stimulation occurred. Thus changes in frontal alpha asymmetry index may be observable despite the variability of the background alpha activity since such

variability equally affect the two hemispheres (e.g. in the transition between sleep and waking). This has important implications in regards to possible future clinical use of such measurements. One may suggest that the change in frontal alpha asymmetry is observable, as long as the brain is capable of information processing as evidenced under anesthesia (Magarey & McCutcheon, 2005; Orser et al., 2007; Sandin et al., 2000) and coma (Boly et al., 2007).

The result of alpha asymmetry found in the present study has three important implications: i) they prove that aversive conditioning (e.g. induced by aversive images and pain stimuli) can be successfully established in wakefulness and its extinction can be obtained during subsequent sleep; ii) they also prove that different types of aversive stimulation during sleep produces a measurable response in brain activity consistent with the implicit processing of negative emotions/distress, regardless of state; iii) this consistency in the asymmetry index throughout different states suggests that such objective response may be similarly obtained in other non-communicative states (e.g. anesthesia or coma) during acute distress thus providing a suitable clinical application for this procedure.

An additional important achievement is the establishment of aversive images as unconditioned stimuli in classical conditioning. This study is unique in analysing the effect of emotional conditioning, obtained using aversive images as unconditioned stimuli, during sleep. The technique proved highly successful both during the acquisition phase in wakefulness and in the extinction phases during sleep.

Since Davidson's findings linked asymmetric frontal activity to negatively valence emotions (Davidson et al., 1979), asymmetry indexes has been an important method in the psycho -physiological study of emotions (Allen, 2004). It is reasonably established that

EEG frequencies in the alpha range are contrariwise related to level of cortical activity in wakefulness (Cook et al., 1998; Marosi et al., 2001). Increased alpha activity may be observed when underlying systems disengage from active processing. Still, other procedures have been used. Many studies have analysed and reported findings in other frequency bands (Aftanas et al., 2006; Marosi et al., 2001), sub bands (Marosi et al., 2002; Rusalova & Kostyunina, 2004), as well as examined other scalp sites (parietal, anterior, central and occipital leads)(Harmon-Jones, 1998). In addition, multiple bands spectral correlations of emotional states have also been considered (Rusalova & Kostyunina, 2004). Nevertheless, many confirms alpha as suitable in studying hemispheric asymmetry (Allen et al., 2004; Marosi et al., 2001). A substantial amount of literature now links alpha asymmetry to motivational or affective style/traits (Jacobs & Snyder, 1996), discrete emotional states (Rusalova & Kostyunina, 2004), the approach/withdrawal model (Coan & Allen, 2003), and increased risk for affective disorders (Davidson, Pizzagalli, Nitschke, & Putnam, 2002). Greater right than left alpha symmetry has been suggested to express positive affect (Tomarken et al., 1992a; Wheeler et al., 1993), greater left asymmetry is related to negative valence affect (Davidson et al., 1990). These propositions are in agreement with the hypothesis made as well as results found in this study. Although it does not exclude that a response may also be understood in terms of a motivational direction.

Davidson's proposition that frontal EEG asymmetries were a function of W/A tendencies instead of emotional valence directed an increased research interest to frontal lobes activity (Coan & Allen, 2004; Davidson et al., 1990; Marosi et al., 2001). There are conceptual similarities between the W/A model and Gray's behavioural systems; BIS, BAS and fight- flight freeze system (FFFS). The FFFS is linked to avoidance behavior.

Approach are mediated by BAS. The BIS, is employed in conflict situations having similarly activating and equally incompatible goals. The BIS facilitates reassessment of alternatives, by inhibiting ongoing behavior and increased focus on negative characteristics in both the conflicting goals (Gray & McNaughton, 2000). An overlap between the approach and BAS is found, but not between BIS and withdrawal. The BIS and BAS have been advocated to better account for frontal hemispheric lateralization in emotional and motivational states (Wacker et al., 2003). Debate still stands as to what exactly is explained with a frontal alpha asymmetry index. Despite these incongruities, frontal alpha asymmetry is an established measurement of emotion and motivation. Studies furthermore indicate that this measurement has a good both test retest as well as internal reliability (Tomarken, Richard, Davidson, & Wheeler, 1992c). This is in agreement with the significant findings across the three experimental conditions in the present study, showing that aversive and distressing stimuli elicit responses observable as frontal alpha asymmetry.

This study looked at responses to distinct aversive stimuli expecting to observe changes in frontal alpha asymmetry. This hypothesis depends on the assumption that a distressful experience, possibly an unpleasant emotion, would be elicited during sleep. Sleep is by definition a non-communicative state during which subjects cannot report how they feel or what they experience (Carskadon & Dement, 1994). This leaves uncertainty about the nature of induced emotions; if the aversive stimuli were properly processed and if there has been any reaction to them. The weight given to the aversive stimuli and the amount of emotional response may vary extensively between subjects (Marosi et al., 2001). It is well established that the experience of stimuli also in wakefulness, depends on an individual's earlier experiences, and other psychosocial factors (Rosenzweig et al., 2005).

Some subjects could have been more angered than distressed by the noxious stimuli. The inversed change in alpha asymmetry observed in subject 2 in group 2 (see appendix II c) may represent anger and approach response in contrast to other subjects in the present study. However, no reports of the experience of the aversive stimuli could be obtained, and this inversed response could be due to other unknown individual factors. Discrepancies between intended emotional induction and the subjects' actual response has been observed (Wacker et al., 2003). However, in these studies emotions were induced using more complex narratives than in the present experimental protocol (Marosi et al., 2001; Wacker et al., 2003). There is no reason to believe that the aversive conditioned and painful stimuli used in this study could elicit any positive valence emotion neither in waking nor in sleep. Thus, it is assumed that if any implicit emotional response was generated, it can only have been of negative valence. With no verbal report of how stimuli were experienced no speculation of the type of aversive emotional response generated could be made. Nonetheless, this study looks at change not direction in asymmetry to noxious stimuli. In fact both directions of asymmetry could be justified as reflecting different types of negative emotions (e.g. anger vs. sorrow). The specification of what negative emotions that has been elicited by the aversive stimuli presented in this protocol goes beyond the purpose and means of this study. Results confirm that a response was consistently generated in the sleeping subjects. In particular, the left > right frontal alpha activity found in all but one subject suggests that emotions close to distress more than anger were probably contingently produced.

The results discussed above were obtained when no distinction was made between sleep stages. EEG during sleep is characterized by periods of high voltage synchronized

activity and periods with activity similar to that of wakefulness but with other elements not consistent with a fully awakened state (Rechtschaffen & Kales, 1968). High cognitive activity like dreaming is not an exclusive marker of REM sleep (Cavallero et al., 1992; Pivik, 1994; Portas et al., 2000). Mental activity is however varied in different stages of sleep. Since S2 and REM sleep represent a more active state than S3 and S4, they were selected in this study. Furthermore, as no significant difference in frontal alpha asymmetry index response between S2 and REM sleep was found, there was no reason to treat the data as two different variables. Also, as brief awakenings did not significantly change a subject's asymmetry, subjects with small epochs of awakenings during stimuli administration were not excluded. One may speculate that it may be possible to find similar responses in yet other non communicative conditions where the brain is capable of information processing (Boly et al., 2007; Magarey & McCutcheon, 2005; Orser et al., 2007; Sandin et al., 2000).

GSR measurements were obtained under the acquisition and extinction phases of conditioning, and were assessed in wakefulness and in sleep respectively. GSR reliably determined that a proper acquisition had been achieved. However, unlike EEG alpha asymmetry, the GSR data showed no contingent effect in the extinction phase during sleep. Consequently, there was no clear correspondence between GSR and EEG data. This may be related to the slow autonomic activation compared to the cortical response (Eriksen, Olff, Murison, & Ursin., 1999) monitored in this study by EEG (2 seconds). The lack of GSR responses during sleep may also be due to a more diffuse, slow and less consistent autonomous response during certain sleep stages (Liguori et al., 2000). Moreover, GSR is associated with more confounds than EEG power measurements.

In addition, it was considered if the aversive stimulation affected sleep latency and fragmentation (regarded as number of awakenings and number of arousals). Evidence suggest that stress in awake state affects both sleep onset and quality (Stoia-Caraballo et al., 2008). As the experimental group conditions differed both before and during sleep the third night, and before the second night (see figure 1), differential group effects across the different nights could be anticipated. However, no effects neither on latencies nor fragmentation were found. With a low number of subjects and large variance within each group, these results are inconclusive. While sleep measurements may be affected by more general stress exposure, EEG alpha power reveals immediate event related change. In this study the transient nature of the stimulation may not have produced long-lasting effects observable in sleep parameters. Hence, the EEG changes appear to be the most suitable measurement of responses to the aversive conditions in this study.

Methodological issues

Difficulties with pain threshold assessment and conditioning

GSR data from the pain curve assessment, acquisition phase and stimuli administration showed that in some subjects acquisition of conditioning did not occur and in others pain threshold was erroneously established (as detailed in the methods section). This can explain why some subjects did not show any difference between CS+ and CS- and others did not respond to pain stimuli (see Appendix II a). Difficulties occurred during pain threshold assessment, for two different reasons. Some subjects were very nervous during the pain threshold situation. They may thus have experienced and reported stimuli as more

painful than they otherwise would. As a consequence the pain stimuli intensity used for the conditioning in group 2 and for painful stimulation in group 3 may not have been strong enough. Hence a nondiscriminatory response to CS+ and CS- was observed in these subjects. Secondly some subjects demonstrated a very high pain threshold, and may not have received potent enough stimuli to make them respond differentially to CS+ or CS-. These observations emerge when comparing the GSR data (from the pain curve, acquisition and extinction phases) to corresponding EEG responses.

In group 2, no significant EEG results were found in 5 subjects. These five subjects displayed an inconsistent pain curve session, in which a threshold was difficult to determine. Similar results were found for the subjects in group 3 who did not show differential EEG responses to aversive or neutral stimuli. Most of these subjects showed either too high or too low responsiveness during the establishment of pain threshold. In both group 2 and 3 there was a clear correspondence between significant change in frontal alpha bands and a well established pain threshold and subsequent acquisition.

Aversive and pain stimuli administration during sleep

An important aspect of sleep is the withdrawal from the environment. Nevertheless, sensory input is processed in the brain of a sleeping person (Carskadon & Dement, 1994). What is sensed during sleep, to what extent and the response is generated, depends both on the nature of the stimuli and on the characteristics and expectations of the individual (Muzet, 2007; Perrin et al., 1999; Portas et al., 2000). The individual response to given experimental condition during sleep is difficult to predict. As both pain stimuli and aversive conditioned stimuli may be seen as important stimuli for the subject, some arousal was to

be expected. Arousals and awakenings have even been seen as a sign of established conditioning during sleep (Wamsley & Antrobus, 2009). Some change in state could be regarded as part of the response due to the experimental conditioning. As already mentioned, awakenings did not change the lateralization index compared to sleeping subjects. Based on this, short periods of awakenings were not excluded from the data.

There seems to be a connection between what sleep stage the subject was in during stimuli administration and the occurrence of the awakenings. There was only one awakening during REM sleep, and considerably more awakenings in S2. However, in the present study, stimuli were lastly administered in REM sleep. This means that more time had passed since the acquisition phase (group 1 & 2) when stimuli were administered in REM sleep. Time delay or extinction does not, however, explain the effect of sleep stage since there was a large individual variance in time of sleep onset, slow wave sleep latency and consequently in the time when the first set of stimuli was administered. Also, group 3 did not undergo conditioning and still differences between stages were found. Most likely the higher number of awakenings in S2 compared to REM sleep is the result of the higher auditory and to some degree sensory threshold present in REM sleep (Bentley, Newton, & Zio, 2003; Rechtschaffen, Hauri, & Zeitlin, 1966).

In the present study, difficulties were experienced regarding what stimuli potency was to be used in stimuli administration during sleep. On the one hand stimuli had to be strong enough to be registered in the altered awareness of sleep. On the other hand it was an aim that subjects would not be fully awakened at the commencing of administration. Furthermore it becomes difficult to predict the individual perception of stimuli if the subject wakes up completely. In this present study, subjects that woke up due to the stimuli

administered were confused and experienced the pain administration as frightening. One person also reported feeling more in pain than during the pain threshold assessment. This may partly be explained by the confusion described. Subjects may not have "come to their senses", thus lacking psychological defenses normally used to cope with noxious stimuli. The situation was also new to them, and may have been experienced as more uncertain. The above mentioned subjects were excluded from the final analyses. This is nonetheless an ethical issue that should be carefully assessed in future research endeavors administering noxious stimuli during sleep.

Conclusion

The aim of this project was to find a measurable physiological response linked to the human experience of distress in conditions where no overt communication is possible. Changes in both frontal alpha asymmetry and sleep quality in subjects exposed to aversive and control stimuli during sleep were assessed.

Results show that frontal alpha asymmetry changes to left more than right activity when subjects are exposed to aversive stimuli compared to neutral control stimuli. The changes observed in frontal alpha asymmetry have important implications. Firstly aversive conditioning can be successfully established in wakefulness and its extinction can be obtained during subsequent sleep. Secondly, this extinction is best observed as changes in EEG, not GSR. Finally, different types of aversive stimulation during sleep produces a measurable change in frontal alpha activity consistent with the implicit processing of negative emotions/distress (e.g. more left than right). In addition, the similarity of the response obtained regardless of state (S2, REM sleep, or wakefulness) suggests that an

objective measurement of distress responses may also be obtained in other non-communicative states (e.g. anesthesia or coma), providing a possibly important clinical application for this method.

Sleep parameters were not significantly affected by the experimental conditions.

Remarks to future studies

It will be of interest to further explore the effects of aversive stimuli both looking at more hemispheric sites as well as looking for possible differential effects in other bands and sub-bands frequencies. This may further inform the development of clinical tools to detect distress in non-communicating patients.

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Appendices

Appendix I Screening tools and questionnaires

Appendix I a, Epworth søvnighetskala

Epworth søvnighetskala

Navn:_____ Dato:_____

Alder (år)_____ Kjønn (Mann = M, Kvinne = K):_____

Hvor sannsynlig er det at du døser av eller sovner i følgende situasjoner, i motsetning til kun å føle deg trett?

Spørsmålene gjelder din vanlige måte å reagere på i den senere tid.

Selv om du ikke har gjort noe av dette i den siste tiden, så prøv likevel å finne ut hvordan situasjonene ville virke på deg. Bruk den følgende skala for å velge det **mest passende tall** for hver situasjon:

0 = ville **aldri** døse/sovne
1= en **liten sjanse** for å døse/sovne
2 = **moderat sjanse** for å døse/sovne
3 = **stor sjanse** for å døse/sovne

Det er viktig at du besvarer hvert spørsmål så riktig som mulig.

Situasjon	**Sjanse for å døse/sovne (0-3)**
Sitte og lese	
Se på TV	
Sitte, inaktiv på et offentlig sted (f.eks. på teater eller et møte)	
Som passasjer på en en-times biltur uten pause	
Legge deg for å hvile om ettermiddagen hvis omstendighetene tillater det	
Sitte og snakke med noen	
Sitte stille etter lunsj (uten å ha inntatt alkohol)	
I en bil, som har stoppet for noen få minutter i trafikken	

TAKK FOR HJELPEN

Appendix I b, Spørreskjema om drømmer

SPØRRESKJEMA OM DRØMMER
(Oversatt fra Dream Questionaire, Schredl 2002)

Hensikten med dette spørreskjemaet er å finne ut om hvor mange drømmer man husker, innholdet i disse og hvordan du selv opplever drømmene. Vennligst fyll ut skjemaet så fullstendig som det lar seg gjøre, selv om du på enkelte punkter kan være noe i tvil om hva du skal svare.

Alder: _____ år. Kvinne ❒ Mann ❒

1. Hvor ofte husker du å ha drømt de siste månedene?

Aldri ❒
Mindre enn én gang i måneden ❒
Omtrent én gang i måneden ❒
To eller tre ganger i måneden ❒
Omtrent én gang i uken ❒
Flere ganger i uken ❒
Nesten hver morgen ❒

2. Hvor ofte er drømmene dine realistiske (virkelige, konkrete)?

Aldri ❒
Noen ganger ❒
Ofte ❒

3. I hvilken grad er drømmene dine kreative?

Ikke i det hele tatt ❒
I liten grad ❒
Middels ❒
Ganske ❒
Veldig ❒

4. I hvilken grad er drømmene dine følelsesladede?

Ikke i det hele tatt ❒
I liten grad ❒
Middels ❒
Ganske ❒
Veldig ❒

5. Hvilken følelsesmessig karakter består drømmene dine av?

For det meste negative ❒
Nøytrale eller balansert ❒
For det meste positive ❒

Appendix I c, Helseskjema

Helseskjema

Navn:

Alder:_____ år

Dato for utfylling:_____

Videregående ☐ Gymnas ☐ Universitet/høyskole ☐

Inntekt?

Kryss av hvis du har/er:

Allergi/astma	☐	Fibromyalgi	☐	Kronisk tretthets syndrom	☐
Gravid	☐	Hypersomni	☐	Hjerte- kar sykdom	☐
Snorker	☐	Insomni	☐	Influensa, forkjølelse	☐
Røyker du?	☐	Kronisk hodepine	☐		

Er du morgen- eller kveldsmenneske?

❒ utpreget morgenmenneske

❒ mer morgen- enn kveldsmenneske

❒ verken eller

❒ mer kvelds- enn morgenmenneske

❒ utpreget kveldsmenneske

Hender det at du har behov for å bevege beina eller armene, vanligvis i forbindelse med ubehag eller ubestemmelig kribling eller mauring i bein eller armer?..... Ja ❒ Nei ❒

Begynner eller øker behovet for å bevege bein eller armer eller de ubehagelige kriblingene når du er i ro, som for eksempel når du ligger eller sitter?..... Ja ❒ Nei ❒

Er behovet for bevegelse eller de ubehagelige kriblingene delvis eller helt borte når du er i bevegelse, som for eksempel når du går eller ved når du strekker deg?..... Ja ❒ Nei ❒

Er behovet for bevegelse eller de ubehagelige kriblingene verre sent på dagen eller om natten enn resten av dagen?..... Ja ❒ Nei ❒

Hva er vekten din (sånn omtrent)?_____kg
Hva er høyden din (sånn omtrent)?_____cm
Når hadde du sist den første dagen av menstruasjon? _____

Har du andre sykdommer/plager?

Hvilke medisner/naturpreparater bruker du?

Har du vært ute og reist over lange avstander den siste måneden? Ja □

I tilfelle hvor og når?

Appendix I d, Drømmedagbok

Kartlegg drømmene dine med drømmedagbok

Hvordan drømmer du?

Utfylling av drømmedagbok i en til to uker er en god og enkel måte å kartlegge drømmene dine på.

Instruksjoner til bruk av drømmedagboken:

Like etter du våkner: prøv å skriv ned drømmen(e) dine så fullstendig som mulig på et eget ark.

Så besvarer du spørsmålene i drømmedagboken. Bruk skalaen i drømmedagboken til å angi hvordan du drømte.

Det er vanskelig å huske nøyaktig hva man har drømt. Når dagboken likevel inneholder slike spørsmål er det fordi man ønsker at du prøver å gi et anslag på innhold av drømmen din. Hvis det har skjedd noe spesielt om nettene, notér ned hva det var (sykdom, telefonoppringing o.l.).

Drømmedagboken fylles ut hver dag.

DRØMMEDAGBOK

Navn: Skjemaet fylles ut om morgenen. Husk å notere dato.

Utfyllingsdato: Eksempel

	30.03.07	Mandag	Tirsdag	Onsdag	Torsdag	Fredag	Lørdag	Søndag
1. Drømte du i natt? 0 = nei, 1 = ja, men husker ikke innhold av drømmen, 2 = ja, jeg husker drømmen	2							
2. Var drømmene dine følelsesladede? 0 = ikke i det hele tatt, 1 = i liten grad, 2 = ganske, 3 = veldig	2							
3. Var drømmen(e) dine.. 0 = hovedsakelig negative, 1 = balansert, 2 = hovedsakelig positive	1							
4. Hvor positiv eller negativ var drømmen(e)? 0 = nøytrale, 1 = litt, 2 = ganske, 3= sterke følelser	1							
5. Hvor realistisk var drømmen(e) dine? 1 = realistisk, 2 = ikke så realistisk, 3 = noen merkelige assosiasjoner, 4 = flere merkelige (bisarre) assosiasjoner	3							
6. Var drømmen(e) dine kreativ(e)? 1 = ikke i det hele tatt, *2* = i liten grad, 3 = middels, 4 = ganske, 5= veldig	3							

Appendix I e, Søvndagbok

Kartlegg søvnen din med søvndagbok

Hvordan sover du?
Utfylling av søvndagbok i en til to uker er en god og enkel måte å kartlegge søvnen din på. I moderne behandling av søvnproblemer benyttes slike dagbøker som hjelp til å stille diagnose, og også til å følge respons på behandling.

Instruksjoner til bruk av søvndagbok:
De to første spørsmålene fylles ut om kvelden før sengetid, mens de andre spørsmålene besvares om morgenen rett etter at du har stått opp. Søvndagboken fylles ut hver dag.
Det er vanskelig å vite nøyaktig hvor lang tid det tar å sovne inn, og hvor lenge man er våken om natten. Når dagboken likevel inneholder slike spørsmål er det fordi man ønsker at du prøver å gi et anslag på disse tidene (ikke se på klokken). Hvis det har skjedd noe spesielt om nettene, notér ned hva det var (sykdom, telefonoppringing o.l.).

Her følger litt hjelp til å fylle ut hvert enkelt spørsmål. Et eksempel på utfylling er også gitt i selve dagboken.

1. *Kvalitet på dagen:* Bruk skalaen i søvndagboken til å angi hvordan du fungerte i løpet av dagen.
2. *Blund:* Alle søvnperioder utenom nattesøvnen noteres, også om blundene var ufrivillige. Hvis du for eksempel sovnet foran fjernsynet i 10 minutter, ønsker vi at du noterer dette.
3. *Hjelp til å sove:* Ta med alle former for sovemidler, også de uten resept. Alkohol-inntak spesielt brukt som sovemiddel noteres også.
4. *Sengetid:* Dette er tiden du går til sengs og faktisk skrur av lysene. Hvis du legger deg kl. 22.45, men skrur av lysene først kl. 23.15, skal begge tidspunktene noteres.
5. *Innsovningtid:* Gi ditt beste anslag over hvor lang tid du tok på å sovne etter at du hadde skrudd av lyset.
6. *Antall oppvåkninger:* Dette er antall nattlige oppvåkninger som du husker.
7. *Varigheten av oppvåkningene:* Angi så godt du kan hvor lenge du var våken i hver av de nattlige oppvåkningene. Hvis dette er umulig, angi cirka hvor lenge du tror du var våken totalt sett i løpet av natten. Ta ikke med tiden det tok fra du våknet til du stod opp, siden det går fram av de neste spørsmålene.
8. *Våkenhet om morgenen:* Her noteres tidspunktet du våknet opp om morgenen uten å få sove igjen. Hvis du våknet kl. 04.00 og ikke sovnet etterpå, noteres dette tidspunktet. Hvis du imidlertid våknet 04.00, men sov en kort periode (f.eks. fra 06.00 til 06.20), noteres 06.20.
9. *Tidspunkt du stod opp:* Her noteres det tidspunktet du stod opp for godt den morgenen.
10. *Søvnkvalitet:* Bruk skalaen i søvndagboken til å angi hvordan du opplevde kvaliteten på nattesøvnen.

Navn: Spørsmål 1 og 2 fylles ut før sengetid, resten av skjemaet fylles ut om morgenen. Husk å notere dato.

1. Hvordan har du fungert i løpet av dagen? 1 = veldig bra, 2 = bra, 3 = verken bra eller dårlig, 4 = dårlig, 5 = veldig dårlig	4							
2. Har du tatt en eller flere blunder i løpet av dagen? Notér tidspunktene for alle blundene.	16-16.30 & 18.15-18.30							
3. Har du tatt sovemedisin og/eller alkohol som hjelp til å sove? Notér medikament og dose, samt evt alkoholinntak	5 mg Imovane							
4. Når gikk du til sengs? Når skrudde du av lyset?	22.30 23.00							
5. Hvor lang tid fra lyset var skrudd av til du sovnet?	45 min							
6. Hvor mange ganger våknet du i løpet av natten?	3							
7. Hvor lange var oppvåkningsperiodene (oppgi antall minutter for hver oppvåkning)?	15, 30, 80							
8. Når våknet du opp om morgenen uten å få sove igjen? Notér tidspunktet for din endelige oppvåkning.	06.15							
9. Når stod du opp?	06.40							
10. Hvordan var siste natts søvn totalt sett: 1 = veldig lett, 2 = lett, 3 = middels, 4 = dyp, 5 = veldig dyp.	1							
11. Hvordan var nattens søvnkvalitet totalt sett: 1= veldig dårlig, 2= dårlig, 3= middels, 4= god, 5= veldig god	2							

Eksempel 30.10.04 Mandag Tirsdag Onsdag Torsdag Fredag Lørdag Søndag

Appendix I f, PSQI

PSQI

Instruksjoner:
Følgende spørsmål har med ditt vanlige søvnmønster *den siste måneden* å gjøre. Du skal svare på hva som er mest riktig for *de fleste* dager og netter den siste måneden.
Vennligst svar på alle spørsmål.

1. I løpet av den siste måneden, når har du vanligvis lagt deg om kvelden?
   VANLIG LEGGETID_____

2. I løpet av den siste måneden, hvor lang tid (i minutter) har det vanligvis tatt deg å sovne om kvelden?
   ANTALL MINUTTER_____

3. I løpet av den siste måneden, når har du vanligvis stått opp om morgenen?
   VANLIGVIS STÅTT OPP KL_____

4. I løpet av den siste måneden, hvor mange timer søvn har du *faktisk* fått om natten? (Dette kan være forskjellig fra hvor mange timer du oppholdt deg i sengen.)
   ANTALL TIMER SØVN HVER NATT _____

For hvert av de følgende spørsmål, kryss av for det beste svar. Vennligst svar på *alle* spørsmålene.

5. I løpet av den siste måneden, hvor ofte har du hatt problemer med søvnen fordi du…

(a) Ikke klarer å sovne i løpet av 30 minutter

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(b) Våkner opp midt på natten eller tidlig om morgenen

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(c) Må opp for å gå på toalettet

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(d) Ikke klarer å puste ordentlig

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(e) Hoster eller snorker høyt

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(f) Føler deg for kald

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(g) Føler deg for varm

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(h) Har vonde drømmer

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(i) Har smerter

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

(j) Andre grunner, vennligst beskriv_____
_____

Hvor ofte, løpet av den siste måneden, har du hatt problemer med søvnen på grunn av dette

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

6. I løpet av den siste måneden, hvordan vil du bedømme søvnkvaliteten din totalt sett?
   - Veldig bra _____
   - Ganske bra _____
   - Ganske dårlig _____
   - Veldig dårlig _____

7. I løpet av den siste måneden, hvor ofte har du tatt medisin (med eller uten resept) som hjelp til å sove?

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

8. I løpet av den siste måneden, hvor ofte har du hatt problemer med å holde deg våken under bilkjøring, måltider eller når du holder på med sosiale aktiviteter?

Ikke i løpet av den siste måneden___	Mindre enn en gang i uken___	En eller to ganger i uken___	Tre eller flere ganger i uken___

9. I løpet av den siste måneden, hvor stort problem har det vært for deg å ha overskudd nok til å få ting gjort?

   Ikke noe problem i det hele tatt _____
   Bare et lite problem _____
   Et visst problem _____
   Et stort problem _____

10. Deler du seng eller rom med noen?

   Deler ikke seng eller rom med noen _____
   Partner/romkamerat i annet rom _____
   Partner i samme rom, men ikke i samme seng _____
   Partner i samme seng _____

Pittsburgh Sleep Quality Index
(Buysse, Reynolds III, Monk, Berman & Kupfer, 1989)
Til norsk ved Petter Franer, Inger Hilde Nordhus, Ståle Pallesen og Simen Øverland

Appendix II Tables and grounds for exclusion

Appendix II a, Overview, Pain curve evaluation and conditioning

Pain curve, acquisition assessment and differential responding to aversive and neutral stimuli Measured in GSR and frontal alpha asymmetry

In group two, no significant EEG results were found in subjects number 14, 17, 26, 29. Three of these subjects (17, 26, and 29) had an inconclusive pain curve session. All had a following vague conditioning phase the subsequent experimental night. Similar results are found in group 3. Here subjects 6, 24, 36, 39 had no significant EEG response to painful stimuli compared to the control sound. Most of them showed either too high (6) or to low (24, 36, and 39) responsiveness during establishment of pain threshold. Here also a correspondence between significant change in frontal alpha bands and a well established pain threshold was observed.

Group1	Subject	paincurve	acquisition	extinction	exrem	alpha
	4		ok	ok	no	opposite
	7		ok	ok	inconclusive	ok
	10		ok	ok	inconclusive	ok
	13		ok/medium	some	some	ok
	16		medium	no	no	ok
	22		ok	ok	some	ok
	25		ok	inconclusive	no	ok
	28		poor	inconclusive	inconclusive	ok
	31					ok
Group2						
	2	ok	ok	no response	no response	opposite
	5	ok	ok	inconclusive	inconclusive	ok
	8	ok	ok	some	some	ok
	14	inconclusive	medium/poor	no	no	no
	17	inconclusive	medium/poor	no	no	no
	20	ok	ok	no	no	ok
	26	inconclusive	medium/ok	opposite	no	no
	29	ok, not great	medium/ok	no	no	opposite
	32	ok, but low	low	no	no	ok
Group3						
	3	ok		no	some	opposite
	6	ok/low		no	no	identical
	9	ok		some	some	no
	12	ok		no	some	ok
	21	ok		some	some	ok
	24	low		no	no	opposite
	27	ok		some	some	ok
	30	low		some	some	inconclusive
	36	too low		no	no	no
	39	too low		opposite	no	no

Appendix II b, Exclusions

* = included in alpha index analyses (n= 27), ** = included in second alpha index analysis (n = 17), x= excluded from sleep analyses

Group	Subject	
1	1	Time codes not found in somnologica
2	2	** x
3	3	**
1	4	**
2	5	**
3	6	Problems in pain threshold assessment*
1	7	**
2	8	**
3	9	**
1	10	**
2	11	Did not complete night 3
3	12	**
1	13	**
2	14	Problems in conditioning protocol*
3	15	did not recieve stimuli in correct sleep stage
1	16	**
2	17	Problems in conditioning protocol*
3	18	completely awaken by pain stimuli
1	19	two standards deviations from all other subjects/ possibly sick
2	20	**
3	21	did not recieve stimuli in correct sleep stage
1	22	**
2	23	did not recieve stimuli in correct sleep stage
3	24	Problems in pain threshold assessment*
1	25	**
2	26	Problems in conditioning protocol*
3	27	**
1	28	**
2	29	Problems in conditioning protocol*
3	30	Time codes not found
1	31	**
2	32	Problems in conditioning protocol*
3	36	Problems in pain threshold assessment*
3	39	Problems in pain threshold assessment*

Appendix II c, Overview, right and left handedness

R = Right handed

L = Left

2	R
3	R
4	L
5	R
6	R
7	R
8	R
9	R
10	R
12	Data missing
13	R
14	R
16	R
17	R
20	R
22	R
24	R
25	R
26	R
27	R
28	R
29	R
30	R
31	R
32	R
36	L
39	R

Appendix III Values used in data analysis

Appendix III a, Log transformed and indexed frontal alpha asymmetry data

Subjects	csps21	csps22	csps23	csps24	csms21	csms22	csms23	csms24
2	-0,4082	-0,2546	-0,1189	0,1799	0,2917	0,2965	1,5985	0,3189
3	1,7314	-1,1027	-0,2269	-0,6129	2,0687	3,1975	-1,5256	-1,2478
4	-0,1918	0,7140	-1,1783	-0,2332	0,1501	0,1914	0,2467	0,0828
5	2,2614	-1,5815	0,2414	-0,0295	-0,5355	-0,0961	-0,2818	0,0909
6	0,0330	0,0476	-1,5784	-0,4529	-0,6034	0,2978	0,2102	-0,7456
7	-0,0783	-0,1555	-1,6977	-0,2440	-3,0919	0,2891	-0,3180	0,7958
8	-0,3566	1,4761	1,1261	-0,6735	-0,1665	-1,6687	1,2134	0,2054
9	0,6290	-0,9748	-1,3542	-0,8506	0,2198	-1,1123	-1,0669	-0,5665
10	-0,1980	0,1360	0,3195	1,1218	0,0663	0,0917	-0,1090	-0,5042
12	0,3983	-0,2044	0,7364	-0,1419	-0,1311	-0,4728	-0,3285	0,1462
13	0,4116	0,0338	-0,1290	0,3040	0,5454	-0,1104	-0,0199	-0,4153
14	0,7742	-0,4615	0,4963	-0,0438	-0,1796	0,1241	0,1967	-0,9105
16	0,0648	-0,2454	2,0384	0,4606	0,7289	0,0542	-0,1759	0,0585
17	-0,2454	-0,7049	1,8638	0,5439	-1,4178	1,4898	-0,1219	0,7868
20	-0,0747	-0,1378	-0,6429	1,0173	-0,1678	-0,5522	-0,6906	0,3485
22	0,1236	0,2010	-0,2671	0,1081	-1,2159	-0,4776	-0,8449	-0,1189
24	1,1789	-0,6735	2,6734	2,0214	0,8070	1,3641	0,2338	-0,0774
25	-0,0584	0,7794	-0,8869	0,0085	-0,7169	0,1412	-0,0267	0,0355
26	-0,9867	0,7087	0,4871	-0,2798	-1,0278	0,8377	0,9150	-0,0698
27	0,1119	-0,5230	0,5439	0,1040	0,1282	-0,4469	0,6408	-0,3659
28	-0,9596	0,7962	-1,0061	-0,0176	-0,3584	-0,3252	0,7301	-0,0211
29	-0,7033	-0,1687	-2,0649	-0,8005	0,0238	0,1686	0,6006	-0,0708
30	0,4541	0,5393	1,4207	0,3305	-1,0899	0,7515	0,0806	0,1799
31	-0,4685	-0,2352	2,0513	1,0873	-0,7879	-0,2944	0,8020	0,9533
32	1,0416	-0,0218	-0,0722	1,2233	0,0182	-0,0643	-0,4735	-0,6258
36	-0,3106	0,5616	0,7066	-0,3568	0,5599	1,2582	-0,5310	0,5157
39	-0,7759	0,2126	-0,4425	-0,0497	0,1355	-0,7680	0,8354	0,7547
Subjects	csprem1	csprem2	csprem3	csprem4	csmrem1	csmrem2	csmrem3	csmrem4
2	0,4417	-0,3736	1,3033	-1,3013	1,0908	0,1539	-0,8254	0,6544
3	-0,0171	0,2180	-0,7106	0,2803	0,9753	-0,8024	-0,5315	-0,6574
4	0,0863	-0,0025	0,0656	-0,5331	0,2229	0,0001	0,0725	0,1335
5	-0,1623	0,2529	0,0848	-0,0219	-0,1408	-0,6037	-0,2871	0,2831
6	0,6629	-0,4888	0,6574	-0,0349	0,6078	-0,0788	-1,3739	1,8514
7	0,0961	0,1605	0,1280	0,0440	-0,4114	-0,1196	-0,3124	-0,3518
8	0,4452	-0,9996	-0,4688	-0,2811	-1,3042	-1,3782	0,5580	0,5084
9	1,3204	-0,5203	1,1526	-0,4050	-0,2874	-0,3754	0,3458	0,2288
10	0,1162	0,0959	0,5890	-0,1338	1,9071	-0,4504	0,4641	-0,0952
12	-0,0241	0,4215	0,2970	-0,6490	2,2210	-1,2636	-2,0656	-0,1185
13	0,5364	-0,3625	0,3368	-0,5246	-0,7188	0,2669	-0,4029	-0,4796
14	-0,9477	-0,1595	0,6945	-0,6231	0,4418	-0,7690	0,1400	0,4512
16	-0,0005	0,0701	-0,3789	0,0072	-0,2084	0,5174	0,0480	-0,5571
17	0,0394	-0,5435	-0,6882	0,0756	0,0813	0,4750	-0,2441	0,4243
20	0,3525	1,1635	0,0484	0,4095	-0,6928	0,5619	-0,6349	0,6573
22	0,4868	-0,0446	-0,4779	-0,1537	-0,2620	-0,0370	-0,3212	0,0544

24	3,4452	0,6776	0,4048	-0,5340	0,0409	0,8528	1,1517	0,7471
25	0,3597	0,0637	0,2451	-0,1687	0,3763	-0,5945	-0,4574	0,1352
26	-0,2543	0,0881	-0,5244	0,6504	0,8128	-0,4465	-0,6955	-0,1228
27	-0,4027	-1,0916	-0,9105	-1,2842	0,1698	0,6518	-1,6623	-0,0759
28	1,0833	0,5794	-0,0895	0,8601	-0,2765	0,1913	0,0991	-0,7892
29	-0,5854	1,1336	0,2873	-0,1814	1,0236	0,4114	1,2818	0,4623
30	0,7917	-0,0236	-1,1019	-0,6503	0,5723	-1,1344	-0,2242	-1,0282
31	0,6440	-0,9368	0,0509	0,7228	-0,1881	-1,1041	-0,6254	0,0136
32	0,1980	0,6311	0,1254	0,4262	-0,7634	1,2879	-0,3617	-0,1311
36	-0,8610	0,8078	1,3554	-0,5781	-0,2361	-0,9240	0,6469	-0,5758
39	0,3617	-1,2097	0,6807	0,3900	-1,5243	-0,6245	-0,1959	-0,2656

Appendix III b, Sleep data

Latencies reported in minutes, arousals, and awakenings reported in total numbers.

Subjects	Latencies Night 2	Latencies Night 3	Awakenings Night 2	Awakenings Night 3	Arousals Night 2	Arousals Night 3
3	10	3	2	5	28	39
4	10	9,5	6	7	57	56
5	16	20	10	2	18	18
7	15	18,5	4	16	68	32
8	16	13	9	8	52	43
9	13	10	4	10	23	17
10	8	4,5	7	10	38	23
12	.	11,5	.	3	.	32
13	7	5	5	3	39	56
16	4	6	5	17	30	38
20	7	7	10	7	30	38
22	18,5	20	7	13	33	40
25	7	8,5	7	8	25	23
27	10	7,5	3	3	49	50
28	10	9,5	13	11	23	27
30	3	2	5	11	8	21
31	16,5	25,5	8	1	17	28
32	36,5	28	6	4	43	52

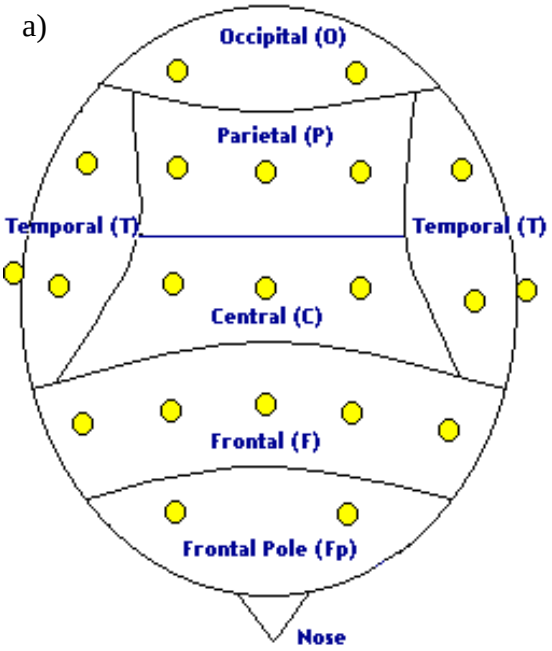
Appendix IV, Abbreviations

Unconditioned Stimuli	UCS
Conditioned stimuli	CS
Withdrawal/approach	W/A
Galvanic skin response	GSR
Polysomnography	PSG
Electroencephalogram	EEG
Electrooccculogram	EOG
Electromyogram	EMG
Stage 1	S1
Stage 2	S2
Stage 3	S3
Stage 4	S4
Rapid eye movement	REM
Non Rapid eye movement	NREM
Restless legs syndrome	RLS
Periodic limb movements	PLM
Periodic limb movements disorder	PLMD
Multivariate analysis of variance	MANOVA
Frontal polar	Fp
Frontal	F
Central	C
Parietal	P
Occipital	O
Auricular	A
Log tranform	Ln
Behavioral activation system	BAS
Behavioral inhibition system	BIS
Fight flight freeze system	FFFS

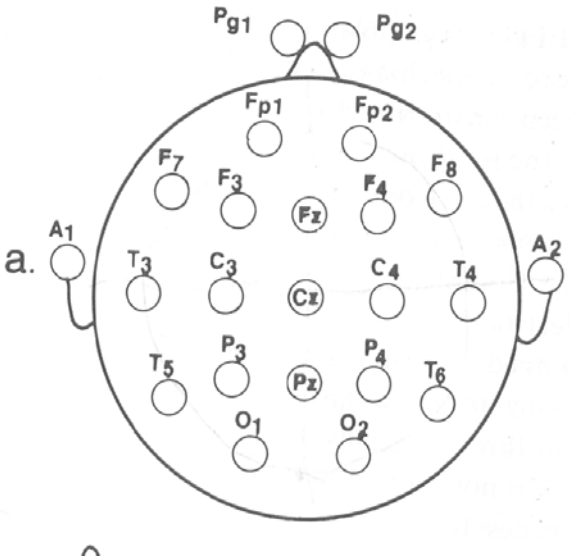
Appendix V, The standardized 10/20 system

The standardized 10/20 system for the placement of electrodes throughout the scalp. The locations are determined by reference points nasion and inion (c). From these points, the skull perimeters are measured in transverse and median planes, divided into 10% and 20% intervals (c). Each position is named according to region placement. Even and odd numbers specify hemisphere and antero- posteriority (e.g. right = even; left = odd) (a,b)

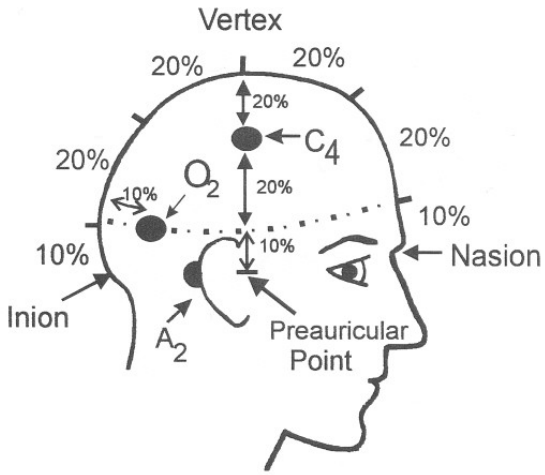
a)



b)



c)



Appendix VI Information sheets given to participants

Appendix VI a Information form

Forespørsel om å delta i forskningsprosjektet ”Betydning av følelser og smerte under søvn”

Prosjektets formål

Hvor mye en person lider, enten følelsesmessige eller fysisk er spesielt vanskelig å forstå hos personer som har svekkede evner til å kommunisere dette (for eksempel dødssyke som ikke er i stand til å snakke, eller hos spedbarn). Slike pasientgrupper kan man si er i en ”stille tilstand” (silent state). For å måle smerte og lidelser ved slike tilstander brukes hovedsakelig ansiktsutrykk. Disse metodene undervurderer ofte smerte og er hovedsakelig nyttig ved tilstander hvor personen er veldig sterkt

I dette forsøket benytter vi søvn som en eksperimentell modell for ”stille tilstand”. Det er kjent at personer som opplever ubehag under søvn (f. eks. mareritt) er uten evne til å kommunisere sin tilstand til omgivelsene.

Formålet med denne undersøkelsen er å se om endringer i hjerneaktivitet, hjertefrekvens, hudsvette og pust kan identifisere responser koblet til følelsesmessig eller fysisk smerte under søvn. Vi vil også undersøke om disse endringene kan kobles til mål på stress og innhold i drømmer.

Beskrivelse av forskningsprosjektet

For deg som forsøksperson vil forsøket innebære at du først møter til noen tester på dagtid. Her måles hvor sterkt du oppfatter følelser knyttet til bestemte ord. For å kunne bli med i undersøkelsen må du skåre over et bestemt minimum på denne ord-testen.

Alle forsøkspersoner som går videre skal fylle ut en søvndagbok i en uke før selve forsøket påbegynnes. I denne perioden må en opprettholde et regelmessig søvnmønster. Du blir også bedt om å beskrive deg selv som person på en personlighetstest samt å fylle ut et spørreskjema om helsetilstanden din.

Deretter skal du sove tre netter på et søvnlaboratorium. Du må ikke innta alkohol de siste 12 timene eller spise eller røyke minst 1 time eller innta kaffe/koffein 6 timer før du møter på søvnlaboratoriet.

De nettene du skal overnatte på søvnlaboratoriet møter du opp kl. 22:00. Her får du påmontert fysiologisk utstyr for å registrere søvnen. Dette utstyret består av elektroder som festes på hodet (7 stk.), nær øynene (2 stk.), under haken (2 stk.) på brystet (2stk), mellom nese og munn og belter (2 stk) over bryst og mage og en på en finger.
Du må avgi spyttprøve før du legger deg og når du står opp. Det skjer ved at du legger en bomullsbit i munnen i minst ett minutt. Bomullsbiten legges så i en oppbevaringssylinder. Spyttprøven skal analyseres for å måle nivået av et stresshormon. Før du legger deg vil du også bli spurt om hvor stresset du føler deg. Du vil bli vekket hver morgen ca kl 7:30. Etter oppvåkning alle netter vil du bli spurt om hvordan du sov, om/hva du drømte, og blir bedt om å rapportere hvor stresset du føler deg.

Den andre natten vil terskelen din for oppvåkning etter smerte og lydstimuli bestemmes under søvnstadium 2. Dette søvnstadiet ligger mellom våkenhet og dyp søvn. Smertestimuli som benyttes er små elektriske støt.

Forsøkspersoner blir for natt 3 delt inn i 2 grupper. Før den tredje natten vil personer i gruppe 1 gå gjennom en læringsprosess. Dette skjer ved at du får presentert et lydsignal. Like etterpå får du et elektrisk støt (fysisk smerte) eller du ser bilder med et ubehagelig innhold (følelsesmessig ubehag). I løpet av den tredje natten vil det samme lydsignalet bli presentert mens du sover i søvnstadium 2. Et helt annet lydsignal (kontroll-lyd) vil også bli presentert mens du sover i søvnstadium 2.

Personer i Gruppe 2 vil ikke gå gjennom denne læringsprosessen. I løpet av den tredje natten vil personer i gruppe 2 bli utsatt for små for elektriske støt med samme frekvens som de i Gruppe 1 fikk under læringsprosessen. Personene i gruppe 2 vil få presentert de samme kontroll-lydene under søvnstadium 2 som dem i gruppe 1.

Hvem kan delta?
Vi søker etter deltakere med normalt god helse i alderen 20 – 40 år

Bivirkninger
Det skal normalt ikke forkomme bivirkninger etter forsøket. Hvis du opplever noen form for ubehag i ettertid kan du kontakte forsøksansvarlig for studien (se nedenfor).

Taushetsplikt
All informasjon vil bli behandlet konfidensielt. Oppbevaringen av opplysningene er regulert av bestemmelser utformet av Datatilsynet.

Eventuell avbrytelse av deltakelse i prosjektet

Du kan når som helst trekke deg fra forsøket uten at dette får noen form for negativ konsekvens for deg og alle innsamlede data vil bli slettet dersom du ønsker det.

Etikk

Prosjektet er klarert av Regional komité for medisinsk forskningsetikk Vest-Norge.

Appendix VI b, Consentform

SAMTYKKEERKLÆRING:

Jeg erklærer med dette at jeg har mottatt muntlig og skriftlig informasjon om prosjektet og sier meg villig til å delta:

_____ _____

Sted Dato Underskrift

Eventuelle spørsmål kan rettes til:

Janne Grønli, PhD, telefon 55 58 60 03
9. etasje i Bygg for Basale Biologifag
E-post: janne.gronli@psybp.uib.no

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